INTERNATIONAL WORKSHOP

ON

"APPLICATIONS OF BIOTECHNOLOGY IN FORESTRY AND HORTICULTURE

Programme and Abstracts

January 14-16, 1988

Nehru Memorial Museum and Library

Teen Murti House

New Delhi, India

Sponsored by:

Department of Biotechnology
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Organised by:

Tata Energy Research Institute
New Delhi, India

International Workshop on "Applications of Biotechnology in Forestry and Horticulture"

Nehru Memorial Museum and Library
Teen Murti House, New Delhi

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Programme

hursday, January 14

.00 - 9.00	Registration	
	INAUGURAL SESSION	
.00 - 9.15	Presidential Address	Dr. S. Ramachandran Secretary Dept. of Biotechnology
.15 - 9.35	Inaugural Address	Prof. M.G.K. Menon Member Planning Commission
.35 - 9.50	Keynote Address	Mr. T.N. Seshan Secretary Ministry of Environment & Forests
.50 - 10.00	Vote of Thanks	Dr. R.K. Pachauri Director Tata Energy Research Institute
0.00 - 10.30	Tea/Coffee	

SESSION I: CURRENT STATUS OF FORESTRY

hairperson:	Dr. M.V. Rao	Indian Council of Agricultural Research New Delhi, India
apporteur :	Dr. Arundhati Mukhopadhyay	Tata Energy Research Institute New Delhi, India
0.30 - 10.55	Forestry in India: Problems and Perspectives	Dr. T.N. Khoshoo Council of Scientific & Industrial Research New Delhi, India

restation: t high ing	Prof. R.L. Mott North Carolina St University U.S.A.	50 - 2.15	In Vitro Strategies for Tropical Fruit Tree Improvement	Dr. R. Tropic Educat Homest
ling and Mass Improvement y	Dr. P.D. Dogra National Botanica Research Institut Lucknow, India	15 - 2.40	Micropropagation of Sandalwood and Mulberry	Dr. P. Bhabha Centre
India	Mr. A.N. Chaturve Tata Energy Resea Institute New Delhi, India	40 - 3.05	Tissue Culture of Ornamental Plants	Dr. L. Indust school Vosken
		.05 - 3.35	Tea/Coffee	
am	Indian Agricultur Research Institut New Delhi, India	.35 - 4.00	Commercial Tissue Culture Propagation of Orchids in Thailand	Dr. Ut Bangko Compan Bangko
	1st Cross Road Ramalinga Road V.V. Layout K.K. Pudur Post Coimbatore, India	.00 - 4.25	Large Scale Production of Woody Plants	Dr. Ro Native Salt L
		.25 - 5.25	Discussion	
	VANARAI People's Movement Green India 2064, Vijay Nagar Pune, India	anelists:	Dr. Robert D. Locy	Native Salt L
			Dr. R.M. Pandey	Indian Hortic Bangal
	Chief Conservator Forests Jaipur, India		Dr. H.C. Chaturvedi	Nation Resear Luckno
	Department of Non- Conventional Ener Sources New Delhi, India		Mr. K.L. Chugh	Bhadra Boards Secund

riday, January 15

LOITATION OF TISSUE CULTURE IN
ICULTURE

SESSION III: APPLICATION OF TISSUE CULTURE IN
HORTICULTURE

a	Indian Agricultur Research Institut New Delhi, India	hairperson:	Dr. V. Jagannathan	Tata Instit New De
	Tata Energy Resea Institute New Delhi, India	apporteurs:	Dr. K. Manoharan & Ms. Abha Agnihotri	Tata Instit New De

9.00 - 9.25	Artificial Seed Production and Forestry	Dr. K. Redenbaugh Plant Genetics Inc. Davis, U.S.A.
9.25 - 9.50	Protoplast Culture and Genetic Manipulation	Dr. Deepak Pental Tata Energy Research Institute New Delhi, India
9.50 - 10.15	Tissue Culture of Palms: Date Palm and Oil Palm	Prof. A.D. Krikorian State University of New York, U.S.A.
10.15 - 10.40	Tissue Culture of Poplars	Mr. G.S. Cheema Tata Energy Research Institute New Delhi, India
10.40 - 11.10	Tea/Coffee	
11.10 - 11.35	Metabolic Phenotypes in Somatic Embryogenesis and Polyembryogenesis	Dr. D.J. Durzan Agricultural Experiment Station Davis, U.S.A.
11.35 - 12.00	Alteration of Growth and Morphogenesis by Endogenous Ethylene and Carbon dioxide in Conifer Tissue Culture	Dr. Prakash Kumar University of Calgary Calgary, Canada
12.00 - 12.25	Storage of Forest Tree Germplasm at Sub-Zero Temperatures	Dr. M.R. Ahuja Institute of Forest Genetics and Forest Tree Breeding Grosshansdorf, F.R.G.
12.25 - 1.25	Lunch	
1.25 - 1.50	Tissue Culture of Plantation Crops	Dr. V.R. Prabhudesai Hindustan Lever Research Centre Bombay, India
1.50 - 2.30	Discussion	
Panelists:	Dr. I.P. Abrol	Indian Council of Agricultural Research New Delhi, India
	Dr. S.S. Bhojwani	University of Delhi Delhi, India
	Dr. H.K. Srivastava	Department of Biotechnology New Delhi, India

2.30 - 3.00 Tea/Coffee

SESSION IV: GENETIC ENGINEERING OF FOREST SPECIES

Chairperson:	Dr. D.J. Durzan	Agricultural Experiment Station Davis, U.S.A.
Rapporteur :	Dr. Shirish Ranade	Tata Energy Research Institute New Delhi, India
3.00 - 3.25	Genetic Manipulation of Woody Species: Prospects and Potentials	Dr. Malathi L.Kumaran Tata Energy Research Institute New Delhi, India
3.25 - 3.50	Direct and Indirect Gene Transformation System in Douglas Fir (*Pseudotsuga-menziesii*)	Dr. Promod K. Gupta National Chemical Laboratory Pune, India
3.50 - 4.15	The Biological and Economic Feasibility of Genetic Engineering for Tree Harvest Index and Wood Properties	Dr. Roger Timmis Weyerhaeuser Company Washington, U.S.A.
4.15 - 4.45	Discussion	
	Prof. R.L. Mott	North Carolina State University, U.S.A.
	Prof. Sipra Guha-Mukherji	Jawaharlal Nehru University New Delhi, India
4.45 - 6.00	Panel Discussion	
Panelists:	Dr. R.D. Lai	Tata Tea Limited, Calcutta, India
	Mr. D.N. Mishra	Amrapali, B-155 Lucknow, India
	Prof. H.Y. Mohan Ram	University of Delhi Delhi, India
	Dr. S.B. Raj Bhandary	Department of Medicinal Plants, Ministry of Forests & Soil Conservation, Kathmandu, Nepal
	Mr. S. Shyam Sunder	Chief Conservator of Forests, Karnataka Bangalore, India

Saturday, January 16

SESSION V: NITROGEN FIXATION STUDIES IN FORESTRY

Chairperson:	Dr. Mahatim Singh	G.B. Pant Universit of Agriculture and Technology Pantnagar, India
Rapporteur :	Mr. Alok Adholeya	Tata Energy Researc Institute New Delhi, India
9.00 - 9.25	Micropropagation and Nodulation of Tree Legumes	Dr. Vibha Dhawan Tata Energy Researc Institute New Delhi, India
9.25 - 9.50	Role of Mycorrhizae in Forestry	Dr. H.S. Thapar Forest Research Institute Dehradun, India
9.50 - 10.15	Investigating the Benefits of Tripartite Association in Actinorhizal Plants for Enhanced Forest Productivity	Dr. Lake Chatarpaul Petawawa National Forestry Institute Ontario, Canada
10.15 - 11.00	Discussion	
Panelists:	Dr. Y.D. Gaur	Indian Agricultural Research Institute New Delhi, India
	Dr. Sunil Khanna	Tata Energy Research Institute New Delhi, India
	Dr. K.G. Mukherji	University of Delhi Delhi, India
11.00 - 11.30	Tea/Coffee	

SESSION VI: THE APPLICATIONS AND POTENTIAL OF BIOTECHNOLOGY IN FORESTRY

11.30 - 11.55	Tissue Culture: Pilot Plant Unit	Dr. R.D. Lal Tata Tea Limited Calcutta, India
11.55 - 12.20	Integrating Biotechnology in Plant Breeding Programmes	Prof. Bruce Haissig USDA Forest Sciences Laboratory Wisconsin, U.S.A.

12.20 - 12.45	Biotechnological Application of Plant Tissue Culture to Forest Species in India	Dr. A.F. Mascarenhas National Chemical Laboratory Pune, India
12.45 - 1.45	Lunch	
1.45 - 3.00	Discussions on Recommendations for Research Priorities	
Chairperson:	Mr. R. Venkatanarayanan	Member-Secretary National Wastelands Development Board New Delhi, India
Co-Chairperson :	Dr. T.N. Khoshoo	Distinguished Scientist (CSIR) Tata Energy Research Institute New Delhi, India
Panelists :	Mr. A.N. Chaturvedi	Tata Energy Research Institute New Delhi, India
	Dr. D.J. Durzan	Agricultural Experiment Station Davis, U.S.A.
	Mr. Samir Ghosh	I.T.C. Ltd. Calcutta, India
	Dr. R.L. Mott	North Carolina State University, U.S.A.
	Dr. R.K. Pachauri	Tata Energy Research Institute New Delhi, India
	Mr. K.G. Venkataraman	National Bank for Agriculture & Rural Development Bombay, India
3.00 - 3.30	Tea/Coffee	

VALEDICTORY SESSION

Chairman :	Prof. R.L. Mott	North Carolina State University, U.S.A.
3.30 - 4.30	Valedictory Address	Dr. Kamla Chowdhry Chairman, National Wastelands Development Board, New Delhi, India

ABSTRACTS

FORESTRY IN INDIA: FUTURE PERSPECTIVES

T.N. Khoshoo, Distinguished Scientist (CSIR), Tata Energy Researc
Institute, 7 Jor Bagh, New Delhi - 110 003, India.

The paper discusses the declining forest cover in relation to th future roles and goals of forestry in India. It enunciates th possible strategies to remedy the situation, keeping in view th widening gap between supply and demand for wood and wood-relate products. It focusses attention on agro-forestry and industria forestry to meet the demand for goods and services of the rura and urban people and industry, as these can no longer be met fro the natural forests. The only way out is raise large-scale man-made plantations. However, to achieve the high level o production/productivity in plantations, the paper points out tha forest trees have to be treated as "tree-crops". Implicit in thi concept is a process of domestication and selection which mus begin forthwith. The paper outlines the concept of "ideotypes" a applied to trees keeping in view specific end-uses. In the end, the paper discusses the relative advantages and disadvantages o Seed-raised HY Plantations. Genetic-evolutionary, environmenta and management implications of the two types have also bee brought out.

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STRATEGY FOR REFORESTATION: HOW ONE CAN EXPECT HIGH RETURNS B SELECTING SUPERIOR CLONES

R.L. Mott, North Carolina State University, U.S.A.

Abstract will be provided at the time of presentation.

* * *

FOREST TREE BREEDING AND MASS CLONING FOR TREE IMPROVEMENT I INDIAN FORESTRY

P. D. Dogra, Head, Biomass Research Centre, National Botanical
Research Institute (CSIR), Lucknow 226 001, U.P., India.

World-wide activity in forestry shows forest genetics to b the most effective component in tree plantation programmes becaus it produces genetically improved trees that show better growth, tree form, site adaptability, wood quality, crop security an product uniformity. In tree improvement genetical an silvicultural techniques are combined both at nursery and at th planting site level (e.g. genotype/site reactions).

The intraspecific genetic variation found within th distributional range of wild tree species forms the basis fo selection work. This includes provenance, race and plus tre selection and their site-performance evaluations followed by progeny tests and advanced generation selective breeding and hybridization work. Genetical contol is exercised through seed (sexual) and vegetative propagules (asexual reproduction). Improved germplasm thus produced is multiplied by the tree breeder through clonal banks, seed zones, seed production areas and seed orchards. Outstanding genotypes are cloned true to parent only on a small scale and mostly as an aid to breeding through vegetative propagation methods. Genetically improved plants for plantations are however required in large numbers. They can be cloned on a large scale by developing a tissue culture micropropagation technique that can produce a large number of genetically stable and uniform trees identical to the elite parent tree.

The situation of tree improvement and mass cloning research in Indian forestry is discussed with particular reference to tissue culture technique.

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AFFORESTATION IN INDIA

A. N. Chaturvedi, Senior Fellow, Tata Energy Research Institute,
90 Jor Bagh, New Delhi - 110 003 (INDIA)

The forests in India, as they survive today have a very uneven and unbalanced spatial distribution; large areas in different parts of the country are bereft of any vegetation. The growing pressure of population both human and cattle has accelerated the process of deforestation. The ameliorative power of forests, though known to people following shifting cultivation, has failed to be effective due to short cycles of forest regrowth. The climatic conditions in India are very favourable to tree growth. Several tree species both indigenous and exotic have been successfully planted in india in afforestation programmes. A matching of the species to specific site conditions is one of the key factors for successful afforestation. There is no miracle tree that can grow everywhere. No tree species harms the environment. Species of low genetic base like Leucaena leucocephala should be avoided in large scale afforestation. Proper spacing and cultural practices are necessary. The ameliorative effects of different species and their rates of growth need to be studied before arriving at rotations of harvest. Protection of afforestation areas from the local people and their domestic cattle is the most difficult aspect of afforestation. Afforestation can succeed with social awakening of the local people coupled with high degree of technical skill. Success of small trials in protected areas, over short periods and laboratory level studies can be extremely misleading.

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LARGE SCALE PRODUCTION OF WOODY PLANTS

Dr. Robert D. Locy, Native Plants Inc., Salt Lake City, U.S.A.

Abstract will be provided at the time of presentation.

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TRIPLOID SYNTHESIS BY FUSION OF MICROSPORE PROTOPLASTS (N) WITH SOMATIC CELL PROTOPLASTS (2N) AND ITS POSSIBLE IMPLICATIONS FOR CROP PLANT BREEDING AND TREE BREEDING

Deepak Pental, A.K.Pradhan, Anil Grover and Arundhati Mukhopadhyay, Tata Energy Research Institute, 90 Jor Bagh, New Delhi-110003, India

We have developed a selection method for production of triploids by fusion of microspore protoplasts (n) with somatic cell protoplasts (2n). The selection method relies upon one of the parents, from which microspore protoplasts are to be isolated, to carry a dominant drug resistance marker and to fuse these protoplasts with WT somatic cell protoplasts. As microspore protoplasts do not divide and somatic cell protoplasts would be sensitive to the drug, on a selection medium containing the drug only triploid hybrids would be recovered. We have tested this selection method by fusing microspore protoplasts (n) of N.tabacum Km+ (2n=4x=48) with wild type somatic cell protoplasts of N.rustica (2n=4x=48). In six different fusion experiments, 96 hybrid colonies were recovered which could grow on a medium containing kanamycin. These colonies were tested for their hybrid nature by PAGE of callus proxidases. The plants regenerated from selected cell lines were analysed for their hybrid nature by IEF of leaf esterases and by DNA-DNA hybridisation using an 18s rDNA fragment as a probe. All the selected plants carried bands characteristic of both the parents. Analysis of organelle genomes using cloned mitochondrial and chloroplast DNA fragments is in progress.

The utility of triploids lies in the production of alien addition and substitution lines which could be subsequently used for bringing about introgression of alien genes into crop plants. This has been done by sexual crosses in wheat, sugarbeet and more recently in tomato by sexual crosses. By protoplast fusion all the post- and pre-zygotic sexual incompatibilities can be circumvented. The utility of this technique for breeding of crop plants and for breeding of tree species will be discussed in this presentation.

* * *

TISSUE CULTURE OF PALMS: OIL PALM AND DATE PALM

A.D. Krikorian, Department of Biochemistry, Division of Biological Sciences, State University of New York at Stony Brook, Stony Brook, New York 11794-4215, U.S.A.

The benefits that can accrue from application of aseptic culture techniques to palms such as African oil palm and date palm etc. are considerable. Here, the challenge is, as in other instances, to develop methods to such a degree that they reflect a high degree of reliability and quality assurance. This means that *in vitro*--derived or--manipulated materials must be grown to maturity under field conditions, and evaluated agronomically. Information from preliminary field trials on palms is just now beginning to emerge. An attempt will be made to review comprehensively the range of available "tissue culture" techniques for these palms and their potential for commercialization. Special emphasis will be given to problem areas and suggestions made for their resolution.

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TISSUE CULTURE OF POPLARS WITH PARTICULAR REFERENCE TO IMPROVEMENT OF POPLARS IN INDIA

G.S. Cheema, Tata Energy Research Institute, 90 Jor Bagh, New Delhi - 110 003, India

Introduction, evaluation and selection of *Populus* species and clones has been carried out for more than three decades in India. Fast-growing clones of *Populus deltoides* which are suitable for growth in the foothills and plains have been selected. Propagation from cuttings being very successful and easy to practice, the question of reproduction from seed has been seldom considered. However, it is now realized that to have healthy plantations, it is necessary to change clones periodically because of their narrow genetic base and also due to degeneration of clonal vigour. Work on breeding of new poplar clones involving different species, namely *P. ciliata*, *P. deltoides*, *P. yunnanensis*, and *P. robusta* have been initiated only rcently. The major objectives of the selection/breeding programmes are to develop clones that are (1) fast growing; (2) insect and disease resistant, and (3) suitable for different agroclimatic regions. Some of the promising new clones of *P. deltoides* are at various stages of testing and are due to be released soon. Tissue culture propagation could help in the first stage multiplication of the selected clones to raise sufficiently large clonal stock to be followed later by multiplication through conventional methods.

Protocols for clonal propagation from selected *P. deltoides* clones (G-3, G-48, D-121, S7C8) and 65/27 using axillary bud multiplication and rooting have been developd and plantlets transferred to pots. Regeneration of shoots from internodal segments could also be achieved. This would, however, need further refinements for optimal use.

Another species of commercial interest *P. ciliata* (Himalaya poplar) is highly amenable to in vitro methods. Tissue Cultur propagation of this species has been accomplished by (a) multiplication from nodal and shoot-tip explants, (b) regeneratio of shoots from leaf discs, and (c) organogenesis in callu cultures derived from leaf, internodes, and roots. The shoot formed by either of these three methods could be rooted, suitabl hardened and transferred to soil. The cultures produced fro young leaf discs have been maintained as longterm cultures fo over eight years now, retaining the capacity of regeneration o plantlets. Recently I have demonstrated that it is possible t induce somatic embryogenesis and plant regeneration from cell suspension cultures induced from calli initiatd from such longter 'leaf' cultures. The plantlets induced by somatic embryogenesi could also be successfully trnasferred to outdoor conditions, Protoplasts isolated from such totipotent cell suspensions could divide to produce few cell colonies. Work is in progress to get sustained divisions and regeneration of plants from protoplast-derived colonies. Micropropagation methods for initia multiplication of superior tree clones are available both in P. *ciliata* and *P. deltoides*. In future it may be possible to d- large-scale multiplication of *P. ciliata* by somatic embryogenesis Our results with cell, tissue and protoplast culture and some o the work done abroad shows that *Populus* species could be used fo genetic engineering provided a necessity is felt to go that way.

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METABOLIC PHENOTYPES IN SOMATIC EMBRYOGENESIS AND POLYEMBRYOGENESIS

Don J. Durzan, Department of Environmental Horticulture, University of California, Davis CA 95616, U.S.A.

The repetitive budding of somatic embryos in cell suspensio cultures of *Prunus* sp. and the cleavage of somatic polyembryogeni masses of gymnosperms provide new opportunities for the study o discreet metabolic phenotypes in morphogenic somatic cells (1,2) Metabolic phenotypes, so far, have focussed on intermedia nitrogen metabolism and are portrayed by 3-dimensional computer assisted graphic methods showing 1) biosynthetic hierarchies o families or *networks* of free amino acids, 2) temporal events i development involving discreet physiological *states* arbitraril defined by current and past compositional behavior and 3) level of metabolites as a percentage distribution or as a flux in state-network map. Maps are correlated to the protein complemen of cells through a series of 2-D gel electrophoretograms and t the activity of synthetic plant growth regulators (3).

Initial attempts to describe metabolic phenotypes ar reviewed for 1) zygotic embryo development in the pistachio, 2 rescued somatic embryos of several *Prunus* sp., 3) the developmen of carpellary tissues, and 4) somatic polyembryogenesis i conifers. For the latter, because cells in the embryonal suspensor masses are heterogeneous, we report attempts t synchronize cell populations through control of the cell cycle. Synchrony of events in more homogeneous cell populations enhance the computer-assisted portrayal of directed gene expression in metabolic phenotypes.

1. Durzan, D.J. (1987) In Cell and Tissue Culture in Forestry. J.M. Bonga and D.J. Durzan eds. Martinus Nijhoff, Vol. 2: 405-439.

2. Durzan, D.J. (1988) In Proc. Symp. Genetic Manipulation of Woody Plants. Michigan State Univ., Plenum Press (in press).

3. Durzan, D.J. (1988) In Plant Growth Regulators: Their role in tree growth and development. S.V. Ross and S. Kossuth eds. Martinus Nijhoff, (in press).

* * *

ALTERATION OF GROWTH AND MORPHOGENESIS BY ENDOGENOUS ETHYLENE AND CARBON DIOXIDE IN CONIFER TISSUE CULTURES

Prakash P. Kumar and Trevor A. Thorpe, Plant Physiology Research Group, Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4 Canada

We studied the production of ethylene and carbon dioxide in tissue cultures of two coniferous tree species, namely, *Pinus radiata* (radiata pine) and *Picea glauca* (white spruce) to understand their roles in growth and morphogenesis in vitro. When cultured under shoot-forming conditions cotyledons of radiata pine produced more ethylene and carbon dioxide than under non-shoot-forming conditions. Growth (fresh- and dry-weights) and shoot formation were enhanced by the accumulation of these gases during the initial stages of shoot bud differentation. Reducing their concentrations by allowing the gases to diffuse out or by absorbing them into chemical traps resulted in reduced growth and shoot formation.

In contrast, when these gases were allowed to accumulate in flasks containing embryogenic suspension cultures of white spruce, growth of the callus and the number of embryos produced were reduced by about 50% over the control, where the gases were allowed to diffuse out. The pattern of accumulation of these gases in the two conifer tissue cultures in relation to morphogenesis is discussed.

* * *

STORAGE OF FOREST TREE GERMPLASM AT SUB-ZERO TEMPERATURES

M.R. Ahuja, Federal Research Centre for Forestry and Forest Products, Institute of Forest Genetics and Forest Tree Breeding, Sieker Landstrasse 2, D-2070 Grosshansdorf, Federal Republic of Germany

The depletion of gene resources is reaching a danger point due to loss of forests for agricultural and industrial use, human settlements, energy, roadways, and environmental pollution, for example "Waldsterben" (dying forests). In the Federal Republic of Germany more than 50 per cent of the forests are dying or are already dead. In countries like India, the forest decline may be more due to irresponsible cutting down of forests than by other causes. Therefore, preservation of germplasm has become an important aspect of the forest tree genetics, breeding, and improvement programs. Broadly speaking, the preservation of the germplasm may be carried out by:

- Storage of clones in clonal orchards, and
- Storage of germplasm (seed, pollen, plants, twigs, buds, bud meristems, tissues, cells) at low temperatures, or sub-zero temperatures.

The present study was undertaken to investigate the differentiation potential of buds from 14 European aspen (*Populus tremula*) and hybrid aspen (*P. tremula* x *P. tremuloides*) clones stored at sub-zero temperatures (0oC, -5oC, -8oC, -18oC and -80oC) for upto two years. At different periods of storage, bud explants were cultured on a screening medium (modified Woody Plant Medium)* to monitor the growth and differentiation of microshoots. Results of these studies show that storage potential of a clone is essentially determined by the storage temperature and the genotype. In another set of experiments, we tested the storage potential of dormant buds and bud meristems of a number of aspen clones in liquid nitrogen (-196oC). The cryopreservation experiments (storage in liquid nitrogen) were carried out with or without cryoprotectants (DMSO, glycerine). The results of the present investigation will be discussed in relation to storage of endangered forest tree species in gene banks.

* * *

APPLICATIONS OF TISSUE CULTURE TO PLANTATION CROPS

V.R. Prabhudesai, Hindustan Lever Research Centre, Andheri (East), Bombay - 400 099, INDIA

Plantation crops are of great help in meeting human requirments for food, spices, timber, medicines and beverages and they also afford protection against soil erosion, floods and help in water conservation. The role of plantations in restoring natural ecosystems by promoting wood lands, afforestation and plantations in degraded forests, marginal lands, cultivable wastelands and fallow lands is of paramount importance. In a developing country like India, it is imperative to increase the area under plantations which can not only help in improving the ecological and environmental balance but can also be a renewable resource of raw material. The ingenuity will lie in blending the traditional breeding methods with new technologies. Tissue culture can act as an important bridge in this regard.

Tissue culture has become a valuable tool for rapid clonal propagation of several economically important plantation crops such as cardamom, coconut, date palm, oil palm, rubber, coffee, teak, cashew, banana, tomarind and pomegranate. Identified high yielding plants can be selectively multiplied on a large scale using the meristem or bud culture method. Producing thousands of plants starting with a single shoot taken from an elite plant is not at all urealistic as has been exemplified in the case of cardamom, teak, banana, tamarind and pomegranate. On the other hand, some of the plants that are not amenable to meristem culture have been successfully multiplied on a large scale through the process of somatic embryogenesis starting with tissues other than the meristem, such as leaf, root or inflorescence. Oil palm and date palm bear a testimony to this. Coconut, belonging to the same palm group, which is an important oil crop of India has proved to be comparatively difficult in the production of plantlets through somatic embryogenesis despite efforts in many laboratories. Efficient methods for multiplication through similar methods have been developed for other plantation crops such as rubber, coffee and sandalwood with relative ease.

Plantations of perennial oil bearing palms such as coconut, oil palm and a wide range of oil bearing trees could be a solution to our vegetable oil crisis that has resulted in a considerable drain on country's foreign exchange resources. As the yields of these crops are 4-5 times higher per hectare as compared to oil bearing annuals, their introduction on a large scale through uniform high yielding plantations would be extremely important in the present context.

Tissue culture methods have been developed for large scale multiplication of foreign exchange earner like cardamom. Such an approach will have to be extended to others like cashew, an important item for export.

Research Needs

Most of the reports describe the methods from embryonic or seedling tissue and not from elite mature plants, the latter being difficult. Concerted efforts will have to be made for developing methods for propagation of such mature identified elite plants in order to have an output superior in quality and quantity. Although a large number of reports exist on establishment of cultures and regeneration, data is meagre on survival of viability of the plants in the field and their performance. Future stress will have to be laid on this aspect.

Another important aspect which requires careful study is the cytological picture of the tissue culture raised plants together with detailed observations in field trials.

Care will have to be taken to avoid single progeny planting and to have genetic diversity in a plantation to ensure greater adaptability.

Tissue Culture techniques can thus overcome the constraints on rates of multiplication and final yields. Besides, application of somaclonal variation technology and somatic cell hybridisation can facilitate seletion of superior genotypes or combination of desirable traits such as high growth rate, higher yields, resistance to diseases or stress.

* * *

GENETIC TRANSFORMATION OF PLANTS

Malathi Lakshmikumaran, Tata Energy Research Institute, 90 Jor Bagh, New Delhi - 110 003, INDIA

Recent advances in biotechnology have made it possible to genetically transform several plant species. Genetic transformation of forest tree species for improvement will be useful as compared to conventional methods of breeding. One of the prerequisites for introduction of foreign genes into tree species will be the development of methods for gene transfer. Many methods have been developed for introduction of foreign genes into crop plants. The most effective method is Agrobacterium mediated gene transfer. In plants a number of specific genes have been transferred using Agrobacterium such as herbicide resistance gene, kanamycin resistance gene, luciferase gene and gene coding for Bacillus thuringiensis toxin. A few tree species have also been transformed using Agrobacterium strain. The notable ones are loblolly pine, sugar pine, cotton and poplar. Genetic transformation of different plant species will be reviewed.

* * *

DIRECT AND INDIRECT GENE TRANSFORMATION SYSTEM IN DOUGLAS-FIR (PSEUDOTSUGA-MENZIESII)

P.K. Gupta, Division of Biochemical Sciences, National Chemical Laboratory, Pune - 411 008, INDIA

Transfer of foreign gene into plants using Agrobacterium tumefaciens as a vector is now well established. Recently it has also been shown that foreign DNA can be introduced directly into plant genome via direct gene transfer into protoplasts without using Agrobacterium. We have developed both the systems for foreign gene transfer into Douglas-fir cells. Tumors have been induced on in vitro grown shoots/seedlings using two strains of Agrobacterium tumefaciens, K12x167 (1). These strains contain derivatives of the wild type Ti-plasmid pTiA6 that contain

Chimeric foreign gene encoding resistance to the antibiotic Kanamycin. These derivatives were constructed by cointegrating plasmid PCGN 562 or PCGN 167 respectively into pTiA6 by homologous recombination. Tumors were grown on phytohormone free medium and all synthesized octopine. Presence of DNA sequence and expression of Kanamycin phosphotransferase activity (APH(3')II) demonstrate the foreign gene transfer and expression in Douglas-fir cells.

Direct gene transfer techniques using electroporation are a viable alternative to the Ti-plasmid system of Agrobacterium. Embryogenesis from protoplasts have been described earlier using embryonal cell suspension culture of Loblloly pine and Douglas-fir (2). Gene construct of the firefly (Photinus pyralis) luciferase structural gene containing the 35 S cauliflower mosaic virus promotor (3) were used in the electroporation experiments. The luciferase gene was successfully introduced into the embryonal protoplasts of Douglas-fir and Loblolly pine by electroporation. Extract from electroporated embryonal protoplasts (24 and 36 hr after introduction of DNA) produced light when mixed with the substrates luciferin and adenosine triphosphate (4). These results show the potential for biotechnology and genetic engineering of conifers which has been considered more difficult than other forest trees.

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THE BIOLOGICAL AND ECONOMIC FEASIBILITY OF GENETIC ENGINEERING FOR TREE HARVEST INDEX AND WOOD PROPERTIES

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Our ignorance of the molecular-level bases of tree growth is now the major obstacle to applying recombinant DNA technology to forests. Two examples show that some important traits may nevertheless be amendable to such technology.

In the first example a theoretical analysis of carbon allocation together with evidence from genetic tests, mutants, and conventional crop breeding, shows harvest index to be a logical trait for genetic engineering in trees. only modest effort may be needed to gain initial entry to the appropriate biochemical path(s). Commercial use of such "Trunkier" trees could occur in a 14-20 year time frame, and could double a sitess carrying capacity. The main risk is one of undesirable side effects on wood properties.

So much more is known about molecular control in our second example, lignin content and composition, that gene isolation work is already underway in some labs. In this case, commercialization of a lower-lignin conifer with more easily degradable hardwood-type lignin might be expected in 10-15 years. The payoff will be higher fibre yield and lower pulp processing costs, with relatively little risk.

These opportunities notwithstanding, biotechnology research at Weyerhaeuser emphasizes low cost systems for the vegetative propagation of superior genotypes produced in conventional tree improvement programs.

* * *

MICROPROPAGATION AND NODULATION OF TREE LEGUMES

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Rapid clonal propagation is not only the final step necessary in order to utilize vegetatively propagated plants that have been genetically engineered, but it is also widely used for increasing the quantity of any selected clone, no matter how developed or selected. A survey of the literature on micropropagation reveals that although the technique has been commercially exploited for herbaceous ornamentals and crop plants, it is still at a developing stage for woody species. According to a rough estimate, only 8 per cent of the species micropropagated so far constitute forest tree species. Further, the success is limited to juvenile tissues and largely limited to those species which are not difficult-to-root *in vivo*, such as poplars and willows.

Our increasing demands of per capita energy consumption and mountaineering population in some parts of the world has resulted in massive deforestation activities. The vicious cycles of floods and droughts that India is facing today, is an expression of the depleting forest cover. To meet our future energy needs it, thus, becomes essential to afforest the areas which are classified as wastelands today. Fast growing, hardy, robust leguminous trees, that enrich soil with bio-nitrogen fertilizer, are the prime candidates for reclaiming such areas. Further, the biomass production per unit area could be increased by selecting the efficient micro-organisms. Over a period of time, the soil would get improved and become suitable for the growth of other tree species.

The research work on tree legumes would be reviewed in this presentation and highlighted by the author's work on Leucaena leucocephala (Lam.), its hybrids and Prosopis species. The study involves developing efficient methods of cloning, selecting efficient microbes (using seed raised plants) and nodulating micropropagated plants prior to their transfer to the field.

* * *

ROLE OF MYCORRHIZAE IN FORESTRY

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Abstract will be provided at the time of presentation.

* * *

INVESTIGATING THE BENEFITS OF TRIPARTITE ASSOCIATION IN ACTINORHIZAL PLANTS FOR ENHANCED FOREST PRODUCTIVITY

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Actinorhizal plants, such as Alnus and Casuarina offer great potential for increasing the productivity of impoverished soils because of their ability to fix atmospheric nitrogen in a symbiotic relationship with the actinomycete, Frankia. The occurrence of mycorrhizae on actinorhizal plants is seen as a hypersymbiosis which could be of added nutritional significance to the system. There is also some concern that the growing use of chemical weed control agents (herbicides) could suppress these organisms and result in long term nutrient depletion. As part of Canada's national biotechnology/bioenergy program, we investigated the tripartite association of Alnus glutinosa, a Frankia strain, and a mycorrhizal fungus, Paxillus involutus in greenhouse and field studies. The effects of a popular forestry herbicide, Roundup [N-(phosphonomethyl) glycine], were also evaluated. Under greenhouse conditions simultaneous inoculation with Frankia and Paxillus involutus stimulated biomass production, nodulation and acetylene reduction in the absence of glyphosate. Glyphosate at 1.5 L/ha stimulated biomass production and acetylene reduction but tempered the influence of the tripartite association. The 9 L/ha glyphosate treatment reduced shoot growth, stimulated root growth and further reduced the impact of the tripartite association. In the field, at the end of one season about 45% of the seedlings failed to survive in the non-herbicide treated plots whereas all survived the 1.5 L and 9 L/ha treatments. Trends were similar to those observed in the greenhouse. In in vitro studies pure cultures of Frankia and Paxillus involutus were treated with different concentrations of glyphosate. At concentrations of up to 10 ppm, neither organisms was affected by glyphosate but their growth was significantly reduced by the 50-1000 ppm treatments. Our results indicate significant enhancement in the performance of Alnus glutinosa seedlings when inoculated with both Frankia and Paxillus involutus. Recommended application rates of glyphosate may not have any negative effects on growth.

TISSUE CULTURE: PILOT PLANT UNIT

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Abstract will be provided at the time of presentation.

* * *

INTEGRATING BIOTECHNOLOGY IN PLANT BREEDING PROGRAMMES

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Integrating breeding and biotechnology may be more important in genetic improvement programmes for woody than herbaceous species because woody species often have long sexual generations. Main focal points for integration are the two primary bases of genetic improvement, selection and genetic recombination. A possibly important but less obvious focus is that of causing phenotypic expression of normally unexpressed traits. In discussing these foci, I will consider and illustrate three broad genetic conditions: 1) desired gene(s) absent in the target species but present in a sexually compatible species; 2) desired gene(s) absent in the target and also in sexually compatible species; and 3) desired gene(s) present in the target but not phenotypically expressed. The examples illustrate that an integrated approach to genetic improvement uses one technology (breeding or biotechnology) to foster the other to the long term benefit of plant genetic improvement.

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BIOTECHNOLOGICAL APPLICATION OF PLANT TISSUE CULTURE TO FOREST SPECIES IN INDIA

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Forest tree tissue culture in India is being carried out in several Universities and Research Institutions. The main thrust of these activities is directed towards micropropagation, using explants both from adult or juvenile embryonic or seedling tissues. The tree species used in these studies cover a wide spectrum which include desert and arid zone trees, leguminous trees and also trees used for timber, fuel, paper and pulp and in rayon industries.

The application of tissue culture to micropropagation of hardwood forest species is still a controversial issue, because (i) of the high cost of plantlet production which is mainly a result of the labour intensive stages of the process and (ii) of the uncertainties regarding the likely gains. This requires careful field evaluation of tissue culture raised plants with respect to growth rates and wood quality depending on the end uses.

The results of small scale outplantings with tissue culture raised plants of *Eucalyptus* spp., *Salvadora persica*, *Tamarindus indica* and *Dendrocalamus strictus* will be presented together with results on the different approaches followed for making the micropropagation processes cost effective and beneficial for use in forestry programmes.

STRATEGY FOR REFORESTATION : HOW ONE CAN EXPECT HIGH RETURNS BY SELECTING SUPERIOR CLONES

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The gain in biomass yield or the gain in product value resulting from some specific quality trait improvement in tree breeding programme can be predicted from heritability estimates for that trait. The gain is available in two approximately equal parts. Only the additive portion is captured in improved seed from breeding programme but the second part, the non-additive gain, can be captured by vegetative propagation of selected individuals.

It is thus possible ot be fairly certain of the amount of gains expected from cloning. However, turning that expectation into a high practical return is dependent on having cloning methods which work reliably on mature parent selections without genotype base. The methods must not introduce new variation and, most importantly, must be sufficiently inexpensive that the initial cost of planting stock does not erase the return over the long harvest cycle. The last requirement for trees places a greater challenge before tissue culture biotechnology than do most other crops. An evaluation of present methods in our programme for *Pinus taeda* will be discussed from this viewpoint. Methods for early trail selection *in vitro* can add to practical returns. Also, an understanding of physiological processes in tree growth can alert researchers to important shortcuts in the cloning process.

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ROLE OF MYCORRHIZAE IN FORESTRY

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The paper highlights the importance of mycorrhizal inoculation in nurseries and afforestation programmes in the forest tree species. The experimental evidence on growth improvement by mycorrhizal inoculation with Pure cultures, nitrogen fixation by ectomycorrhizal fungi/seedlings, Host/fungus specificity, Rhizobia-VAM interactions are discussed. The potential of mycorrhizal technology as an aid to produce quality planting stocks with efficient mycorrhizal root systems for critical planting sites has been briefly dealt. Priority areas in mycorrhizal research for further detailed investigations are outlined.

Key words : Mycorrhiza, Symbiosis, Inoculation, Afforestation,
Survival, Pisolithus tinctorius

PILOT PLANT TISSUE CULTURE UNIT

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(Abstract of paper presented at the International Workshop on Applications of Biotechnology in Forestry and Horticulture, sponsored by Tata Energy Research Institute, New Delhi, 11-16th January, 1988).

As part of the national goals in the sphere of Energy Conservation and Afforestation, Tata Tea Limited has embarked upon a Biotechnology programme for the generation of fuelwood planting material. The South Indian Tea Industry, and particularly Tata Tea Limited, is replacing the use of fossil fuels in the process of tea drying, and extensive Energy Plantations are required to meet this objective.

A small Pilot Plant has been set up in the Company's tea gardens in Kerala. Relying entirely on indigenous equipment, the micropropagation protocols for three Eucalyptus species, which are particularly suited to our agro-climatic conditions have been standardised. The plants have been transferred to the soil in the nursery, and thence to the field. Studies are now under way to optimise the economics of production.

It is recommended that Plantation Companies be encouraged to develop Energy Plantations. Besides the ecological benefits of these schemes, such organisations, with their experience of managing vast tracts of land and organising large numbers of workers, should be capable of generating revenue and employment as well.

Tata Tea Limited's tissue culture laboratory is also undertaking in vitro studies on Camellia sinesis.

It is hoped that the experience and work carried out at our pilot plant unit will be able to contribute effectively and economically to the country's afforestation projects.

CLONAL PROPAGATION OF PLANTS BY TISSUE CULTURE : POTENTIAL APPLICATIONS IN FORESTRY

Technical Background Note prepared
for International Workshop on

"APPLICATIONS OF BIOTECHNOLOGY IN FORESTRY AND HORTICULTURE"

Jan 14-16, 1988
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Sponsored by :

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CLONAL PROPAGATION OF PLANTS BY TISSUE CULTURE : POTENTIAL APPLICATIONS IN FORESTRY

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Preface

Plant Tissue Culture is an essential component of Plant Biotechnology. It provides means to multiply and regenerate novel plants from genetically engineered cells. The promising individuals thus produced may be cloned in cultures under disease-free conditions. Additional advantages of tissue culture propagation of plants, popularly called micropropagation, are economy of time and space, and freedom from seasonal constraints.

This article, besides introducing the art of tissue culture and micropropagation in particular, also evaluates the current status of higher plant propagation through tissue culture. The potential of this technique forest species, is yet not fully exploited. The importance of clonal forestry and how aseptic techniques can benefit the yields from our forests are highlighted.

The background note is essentially an extention of the author's earlier publication :

Dhawan, V. and Bhojwani, S. S., 1986. Micropropagation in Crop Plants. Glimpses in Plant Research, Vol. 7: 1-98.

Vibha Dhawan
December 1987

1 PLANT TISSUE CULTURE

Plant Tissue Culture' is a blanket term for protoplast, cell, tissue and organ culture under aseptic controlled conditions of light, temperature & humidity, etc. The advancements made in this field are due to the unique capacity of living cells in a plant body, to give rise to whole plants (totipotency) Tissue culture one one hand has become an invaluable aid in the field of experimental botany as it allows us to study basic problems related to growth and differentiation under controlled reproducible conditions On the other hand it has acquired many practical applications in horticulture and agriculture

Before we start on the details of aseptic cloning which is one of the fields where tissue culture is exploited commercially, it is important to be familiar with terminology and methodology of the science; both for maintaining a genotype or generating superior plant types

1 1 Terms commonly used in Plant Tissue Culture (Definitions)

Adventitious Adjective used to describe roots, shoots, or embryos developing from abnormal positions, e.g., shoots from roots, leaves or callus; roots from leaves or callus, and embryos from any cell other than the zygote

Apical dominance· A phenomenon commonly observed in nature, of suppression of growth of axillary buds in the presence of the terminal bud on the branch.

Aseptic culture: Raising cultures from a [illegible] or an organ after freeing it of bacteria, fungi and other microorganisms

Asexual propagation Plant multiplication by using a vegetative tissue

Auxins A class of growth regulators [illegible] cell elongation, apical dominance [illegible] Indoleacetic acid (IAA), naphthalene [illegible] (NAA), Indolebutyric acid (IBA), and [illegible] acid (2,4-D) are some of the [illegible] in tissue culture

Axillary Developing in the axil [illegible] axillary bud

Batch culture A suspension culture in which cells grow in a finite volume of nutrient medium and follow a sigmoid pattern of growth

Callus A tissue arising from disorganized proliferation of cells either in cultures or in nature. Plural calluses/calli

Cell culture Culture of single cells or [illegible] group of similar cells

Chemostat An open continuous culture in which growth rate and cell density are held constant by a fixed rate of input of growth regulators and nutrient

Chromosome Thread-like structures found in the nucleus, which are composed of nucleic acids and proteins. They carry genes and are involved in the transmission of hereditary characteristics

Clonal propagation Asexual plant multiplication starting from a single plant

Clone· A population of cells derived from a single cell by mitotic division It is also used to denote a population of plants derived from a single individual through vegetative propagation

Culture Growing cells, tissues, plant organs, or whole plants in nutrient medium under aseptic, controlled conditions e g anther culture, cell culture, embryo culture, shoot-tip culture, etc

Cytokinins A class of growth regulators responsible for cell division, cell differentiation, shoot differentiation, breaking apical dominance, etc Some of the cytokinins used in tissue culture are benzyl-aminopurine (BAP), 2-isopentenyladenine (2-ip), furfurylaminopurine (kinetin) and zeatin

Differentiation The development of different physiological and/or morphological characteristics For example, organ development in a multicellular organism from a single-celled zygote In tissue culture, the term is commonly used to describe the formation of different cell types, roots, shoots, embryos, or other organ in the callus or cell culture

Diploid Having two copies of each chromosome characteristic for the species

Dominant gene/character· One that is expressed in a diploid cell/organism irrespective of the gene present on homologous chromosome.

Embryo A very young plant developing inside female gametophyte usually formed as the result of fertilization but rarely without fertilization (apomixis)

Embryogenesis: The process of embryo initiation and development

Embryoid: Non-zygotic embryo formed in culture

Excise To remove a piece of tissue or an organ from its parent source, such as, removing shoots from callus, separating individual shoots from a proliferating mass of shoots or cutting single node segments for further proliferation from a shoot

Explant: A plant organ or piece of tissue used to initiate a culture

Gamete: A sex cell derived from a gametophyte and containing one-half the number of chromosomes present in the somatic cells of the plant

Gene: One of a set of units of heredity, having a specific effect on the characteristics of an organism Genes are composed of deoxyribose nucleic acid (DNA) and are arranged linearly on the chromosomes

Genotype The genetic make-up of an individual as determined by the set of genes carried in the chromosomes

Habituation: Ability of cells to grow in the absence of growth regulators This ability is acquired by some cells after prolonged cultivation in the presence of hormones

Haploid Having single copy of each chromosome in a cell. Characteristic of gametic cells.

Heterogeneous population Non-uniform; containing more than one type

Heterozygous Having different alleles at one or more corresponding loci in homologous chromosomes Self-fertilization of heterozygous individuals (cross-pollination) give rise to heterogeneous population.

Homogeneous population Uniform, all of the similar genetic make up

Homozygous Diploid or polyploid individuals having identical alleles on the homologous chromosomes Self-fertilization of homozygous individuals gives rise to a homogeneous population

Hybrid· An organism or cell line resulting from a cross between parents that are genetically unlike (dissimilar)

Hybridization Any process by which hybrids are created (formed)

In vitro Literally "in glass", now applied to any process carried out in sterile cultures.

In vivo Literally "in life" applied to any process occurring in whole organism (in nature).

Induction To cause initiation of a process or a structure

Inoculum· Cells, tissues or organ used for sub-culture, this term is commonly used for aliquot of a suspension culture

Internode· The portion of stem between two nodes.

Meristem A localized group of actively dividing cells, from which permanent tissue systems (root, shoot, leaf, flower) are derived. The main categories of meristems are apical meristems (in root and shoot tips), lateral meristems (vascular & cork cambiums), and intercalary meristems (in the nodal region and at the base of certain leaves).

Meristematic Having the characteristics of a meristem

Meristemoid A localized group of meristematic cells that arise in the callus and may give rise to roots and/or shoots

Micropropagation Asexual or vegetative propagation of plants under aseptic conditions

Morphogenesis The development of form or structure

Nitrogen fixation The conversion of atmospheric dinitrogen (N_2) into ammonia and amino acids

Node· A region on the stem from where a leaf bearing an axillary bud arises

Nutrient medium A semi-solid or liquid combination of nutrients and water, usually including several inorganic salts, a carbohydrate, organic salts and vitamins Such a medium is referred to as basal medium and may be supplemented with growth hormones and occasionally, with undefined substances such as coconut water and malt extract

Organ Culture Aseptic culture of organized structures, e g root tip, shoot tip, shoot segments, embryo, etc

Organized tissue Composed of regularly differentiated cells

Organogenesis: The formation of organs

Plantlet A small rooted shoot or germinated embryo

Ploidy The number of full chromosome sets per nucleus

Polyploidy Containing three or more sets of chromosome per nucleus (triploid, tetraploid, pentaploid, etc.).

Protocorm In orchids, seeds contain an unorganized embryo comprising only a few hundred cells. During seed germination the embryo first forms a tuberous structure called a protocorm, from which develops a complete plant. In cultures, vegetative parts of several orchids form round, glistening, protocorm-like structures which may be multiplied indefinitely or induced to regenerate whole plants

Protoplasts. Single cells with their walls stripped off.

Recessive gene/character: One that is expressed in a diploid cell of an organism only when both sets of chromosomes carry the same gene or genes involved

Shoot tip/Shoot apex Terminal 0.1-1.0 mm portion of a shoot comprising the meristem (0.05-0.1 mm) together with primordial and developing leaves and adjacent stem tissue

Somatic Refers to vegetative or asexual part or process

Sub-culture Sub-division of a culture for transfer to fresh medium.

Suspension culture Cell or cell aggregates cultured in liquid medium

Synchronous culture: A culture in which the cell cycles (or a specific phase of the cycle) of a proportion of the cells (often a majority) are synchronous

Tissue culture. A broad term referred to all types of aseptic plant or animal culture. In case of plants PTC is used as a blanket term for protoplast cell tissue and organ culture under aseptic conditions

Totipotency Potentiality of a single cell to produce a whole organism

Transfer: The process of relocating culture tissue to fresh medium

Vegetative propagation: The asexual propagation of plants by detachment of some part of the plant body, e g a cutting and subsequent development into a complete plant

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Source Bhojwani, S S. and Razdan, M K , 1983. Plant Tissue Culture Theory and Practise Elsevier Science Publishing Company, Amsterdam, pp 520.

1 2 LABORATORY REQUIREMENTS AND GENERAL TECHNIQUES

The size of a tissue culture set up and the extent to which it is equipped, are governed by the nature of the project undertaken and the funds available However, a standard tissue culture laboratory should provide facilities for

a) Washing and storage of glassware, plasticware and other labware

b) Preparation, sterilization and storage of nutrient media

c) Aseptic area for the manipulation of plant material

d) Maintenance of cultures under controlled conditions of light and temperature

e) Space for observation of cultures

At least two separate laboratories or rooms should be available, one for glassware washing and storage and media preparation (media room) and second a culture room to store cultures The culture room should contain a culture observation table provided with binoculars and adequate light source Depending on the local conditions the sterile transfer cabinet (Laminar Air Flow) may be housed in the culture room, in a quite corner of an ordinary research laboratory, or a specially designed transfer room

Requirements for Media Room

Usually the washing machine and large sink with running hot and cold water is adequate for the purpose. Glassware/plasticware is soaked in plastic buckets with soap solution, washed and then rinsed with distilled water. They are then dried in ovens and stored in dust-proof cabinets.

The facilities required for the preparation of the medium include (a) benches of the height suitable to work standing, (b) a deepfreeze for storing stock solution, (c) a refrigerator to store various chemicals, (d) plastic carboys for storing distilled water, (e) weighing balance, (f) a hot plate-cum-magnetic stirrer for dissolving chemicals, (g) a pH meter, (h) an aspirator or vacuum pump to facilitate filter-sterilization, (i) a steamer for melting agar, and (j) an autoclave for media sterilization.

Different types of vessels have been used to culture plant material. While in some cases the choice of culture vial is dictated by the nature of the experiment, in others it has been guided mainly by the convenience of the worker and the laboratory set up for maximum space utilization. For standard tissue culture work glass test tubes have been widely employed. Wide mouthed bottles of different sizes and milk bottles have also been used specially in micropropagation. In tissue culture work only borosilicate or pyrex glassware should be used. Soda glass may be toxic especially after repeated use. For protoplast work normally steriled disposable plasticware is used.

The room for incubating cultures is maintained at a controlled temperature. Usually air-conditioners and heaters are used to maintain the temperature around 25±2 °C. For exploratory experiments (to determine optimum temperature requirement of the species) incubators with built-in fluorescent lights are also used.

Cultures are generally maintained in diffuse light (less than 1 klux). However, in a normal culture room provisions are made for maintaining cultures in higher light intensity as well as in total darkness. Diurnal control of illumination is achieved by using automatic time-clocks. Normally illumination is provided by fluorescent tubes.

The culture room is provided with specially designed shelves for storing cultures. They are normally made up of iron, sometimes also of wood and each shelf is provided with a separate set of fluorescent tubes. While flasks, jars and petri-dishes can be placed directly on the shelf or trays of suitable sizes, to support culture tubes some sort of racks are required. Metallic wire racks, each with a holding capacity of 20 to 24 tubes are suitable for this purpose. On one side of the rack a label giving details of the experiment (name of the plant, explant, medium, date of culture and name of investigator) is stuck.

It is desirable to have emergency power points attached to a generator to maintain both light and temperature in the culture room, and also to eliminate the risk of suspension cultures dying due to stopping of shakers in the event of a major power breakdowm at the mains.

Plant tissue culture media which contains a high concentration of sucrose supports the growth of many microorganisms which on reaching the media grow faster than the culture tissue and finally kill it. Therefore, it is essential to maintain a complete aseptic environment inside the culture vessel. For this, precautions should be taken to remove the contaminated cultures from the culture area as soon as possible and before discarding autoclave them in order to kill microorganisms. Since there are several possible sources of contamination of the medium such as (a) the culture vessel, (b) the medium itself, (c) the explant, (d) the environment of the transfer area, (e) instruments used to handle plant material during inoculation and sub-culture and (f) the environment of the culture room. The microbial contaminants are normally present in the medium right from the start. To destroy them, the mouth of the culture vial containing the medium is properly closed with a suitable bacteria-proof closure and the vial is autoclaved (steam heating under pressure) at 1.06 kg per cm^2 ($121^\circ C$) for 15 minutes from the time the medium reaches the required temperature.

Some growth factors such as GA_3, zeatin, ABA and certain vitamins are thermolabile and cannot be autoclaved. When using such a compound the whole medium minus the heat labile compound is autoclaved in a flask and kept in sterilized hood to cool down. The solution of the thermolabile compound is steriled by membrane filter and added to the autoclave medium when the later has cooled to around 40^{o} C in semi-solid medium (just before the setting of agar) or to the room temperature when using liquid medium. For filter sterilization of the solutions, bacteria-proof filter membranes of the size 0.4 m are used (microfilters).

The instruments used for aseptic manipulation such as forceps, spatulas and scalpals are normally steriled by using 95% ethanol followed by flaming and cooling. This is done several times during the operation. The aseptic manipulation is normally carried out in laminar air flow cabinets. Essentially, laminar air flow cabinet has a small motor to blow air which passes through the coarse filters where it loses large particles and subsequently through a fine filter. The later known as the high efficiency particulate air (HEPA) removes particles larger than 0.1 um. The ultraclean air (free of microbial contaminants) flows through the working area. The velocity of the air coming out of the fine filter is 27±3 m per minute which is adequate for preventing the contaminants of the working area by the worker sitting in front of it and aseptic environment is maintained in the working area as long as the cabinet is kept on.

The other aspects of sterilizing the plant material are discussed in the later section.

2. PLANT PROPAGATION AND MICROPROPAGATION

2.1 Plant Propagation (Sexual vrs Asexual Reproduction)

Plants can be propagated either vegetatively (asexual) or by seed (sexual).

- In the sexual cycle new plants arise after fusion of the parental gametes and develop from seeds. In cross-pollinated plants all the seedlings are genetically variable and each one represents a new combination of genes brought about by meiosis (during the formation of gametes).
- By contrast in the asexual cycles (vegetative) the unique characters of any individual plants mother plants are perpetuated by growth and multiplication of cells in which genes are copied exactly at each mitotic fusion. The plants produced by this method are exact replicates of the mother plants and such a group represents a **clone**.

In few genera plants, genotypically identical to the parents are obtained from seed produced through **apomixes** (seed development without meiosis and fertilization) Apomixis, however, is restricted to only a few species and therefore, commercial nurseries have adopted the methods of vegetative reproduction for multiplying selected cultivars.

In the natural environment sexual and asexual reproduction have their own advantages depending on the species. Seeds may be stored for long periods without loss of viability and are often produced in such large numbers that they are inexpensive. Further, in some plants that are predominantly self-pollinated, seeds give rise to homozygous populations. However, in most plant species seeds are developed from union of gametes of dissimilar parents (cross-pollinated) and hence, their quality depends on the constituent of both the parents. Thus, the seed-derived progeny is dissimilar and represents variability. In many plants there is a long undesirable juvenile phase and viable seeds are produced very late in the life cycle. Therefore, to grow plants through seeds of such species may not prove practical for new field planting and in such cases vegetative propagation is the only means of propagating plants.

The technique of cloning plants is not new. In fact, today most of potato, sugarcane and few fruit trees like apple and mango are propagated only vegetatively through traditional macropropagation techniques. The normal practice is to take a cutting from the plant, apply water mists to prevent desiccation and put them in rooting composts in vivo and finally transferring them to the field. In case of potato and sugarcane they are cut into pieces, each with the axillary bud which sprouts and produced shoot on one end and root on the other.

Whether the plant is propagated by seed, traditional vegetative techniques of tissue culture not only depend on the plant species but also on the agronomic objectives. The potato plants are to be raised from seed during progeny programmes to select new varieties. Tissue culture proves useful to multiply certain lines during a breeding programme and to propagate disease free stock of the established cultivars and macropropagation using field cloned tubers to provide normal planting material.

2.2 Advantages and Disadvantages of in vitro methods

The in vitro methods of cloning plants are largely an extension of those already developed for conventional propagation. These techniques have the following advantages over traditional methods:

1) Cultures are started with very small (explants) and thereafter shoots produced in cultures are propagated (hence the term micropropagation). Therefore, this also implies that a small amount of space is required to maintain and multiply large number of plants.

2) Propagation is carried out under aseptic conditions, free from pathogens. Also, by meristem shoot-tip culture, pathogens like viruses could be eliminated. Therefore, it proves to be a useful method for virus elimination and maintenance of plants in pathogen-free state.

3) Traditional vegetative methods of propagation are not available for all plant types. Under aseptic conditions, adjustment of factors influencing vegetative regeneration is possible such as

manipulating nutrient and growth regulator level, light & temperature, etc. In addition, even for the species where conventional vegetative propagation methods are available, tissue culture proves to be much faster and thus enables newly selected variety to be made available quickly.

4) Production can be continued all the year round irrespective of the seasonal variation.

5) Once the method is standardized the cultures need no attention between subsequent sub-cultures, in contrast to the traditional methods where continuous watch is required for watering, weeding, spraying fungicides, insecticides, etc.

The main disadvantage of in vitro method is that advance skills are required for the successful operation. First of all the initial cost for the set up of the lab is quite high. For each plant species and sometimes even for a variety, specific methods are necessary to be developed to obtain optimal results. Since the whole process is labour intensive the cost of the propagules is relatively high.

Also the test-tube-propagated plants are more delicate than the seedling plants because they grow on sucrose rich medium (therefore, photosynthetically inefficient) and humidity inside the culture vial being high, the plants are equipped with poor mechanisms for controlling water loss.

2.3 Propagation in vitro (Micropropagation)

General interest in the use of tissue culture for clonal propagation of plants originated from its grand success with orchids, the credit for which goes to a French scientist George Morel (1964). While attempting to raise virus-free plants of Cymbidium by culturing less than a millimetre long shoot-tips from infected plants of the orchid, he observed that these 'microcuttings' instead of developing into leafy shoots formed spherule-like structures with rhizoids at the base. These structures were identical to the protocorms (original plantlets) normally formed by embryo during seed germination. Each shoot-tip produced a clump of about half-a-dozen protocorms. If the proliferating mass of protocorms, before the formation of plants, was sliced into three to six pieces and placed on fresh medium, each one grew and budded off into four to eight protocorms within a month's time. This process of protocorm multiplication could be repeated indefinitely. If, however, the protocorms were not chopped, each one of them developed into a plantlet. Morel estimated that even if each protocorm gave only four new plants in a month, it should be possible to raise over four million plants in a year, starting from a single shoot-tip. Appreciative of the great potentials of this new technique, the commercial orchidologists rapidly adopted the aseptic method of cloning orchids. The methodology developed by Morel has been further refined over the years. Practically, for all economically important orchids, techniques of cloning through tissue culture are available. Indeed tissue culture is currently the only economically feasible method for clonal propagation of orchids. All major orchid growers the world over, now have well established tissue culture laboratories.

The technique is further extended to many horticultural species on a commercial scale. According to one estimate, on a world wide basis, 60 million plants are annually produced through tissue culture. It includes such species as oil palm which cannot otherwise be vegetatively multiplied. In India, considerable economic benefits can be derived by evolving efficient micropropagation methods for cloning elite individuals of important crops, such as coconut, cashew nut, date palm and mango apart from species like Eucalyptus where tremendous variability in terms of yields is reported.

3. GENERAL TECHNIQUES OF MICROPROPAGATION

Micropropagation generally involves four steps: (i) Initiation of aseptic cultures, (ii) shoot multiplication, (iii) rooting of in vitro formed shoots and (iv) transplantation of plants to potting mix. Fig. (1) Debergh and Maene (1981) suggested, that another stage zero should be included i.e. mother plant selection and preparation. Further the shoots should be uniformly elongated to have maximum rooting (Fig. 2). Each step has its requirements and are described in detail below.

Stages in micropropagation

Murashige (1974)	Debergh & Maene (1981)
St. I : Initiation	St. 0 : Preperation of the mother plant
	St. I : Initiation
St. II: Multiplication	St. II : Multiplication
St. III: Rooting in vitro	St. III: a. Uniform elongation (preperation for root- in vivo)
	St. III: b. Rooting in vivo

Fig. 2 : Adaptions to the Murashige in vitro multiplication scheme (Debergh & Maene, 1986)

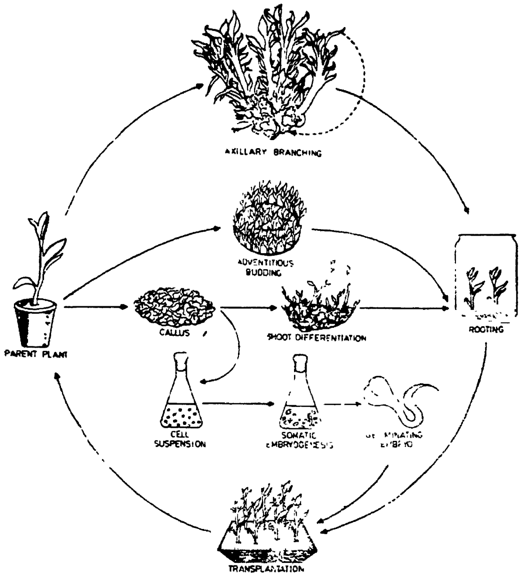

Fig. 1 : Diagrammatised summary of steps involved in aseptic multiplication of plants. Shoot multiplication is achieved through enhanced axillary branching, adventitious budding from pieces of plant parts or callusing. The shoots are individually rooted in a medium containing a suitable auxin. When a satisfactory root system has developed the plantlets are transferred to a well drained potting mix. After maintaining them under high humidity and low light intensity for 1-2 months the plants are transferred to ordinary glasshouse conditions or to the field. Plant multiplication involving callusing may occur either through shoot differentiation or somatic embryogenesis. In the latter case a special treatment for rooting is not required because the embryos have a pre-formed root primordium.

3.1 Stage 0: Mother Plant Selection and Preparation

It is important to select the mother plant and make sure that it is disease free. The field grown plants normally have severe contamination problems and it is important to treat the mother plant/protect the fresh growth from air-borne contaminants. If the mother plant is infected by a virus it is best to initiate the cultures with small shoot-tips.

3.2 Stage 1: Initiation of Cultures

(i) Explant: The nature of the explant to be used for in vitro propagation is, to a certain extent, governed by the method of shoot multiplication to be adopted. For enhanced axillary branching only such explants are suitable which carry a pre-formed vegetative bud. When the objective is to produce virus-free plants from an infected individual it becomes obligatory to start with meristem tips (0.5-1.0 mm comprising of meristematic dome with 1-3 leaf primordia). However, if the mother plant is disease-free the most suitable explant is nodal cutting.

Nodal segments are both easy to obtain and do not require any dissection. Also they withstand the toxic effect of sterilizing agents much better than the terminal cuttings. However, Miller and Murashige (1976) used terminal buds to initiate cultures of *Cordyline terminalis* and *Dracaena godseffiena* (ornamental plant) because lateral buds did not produce reculturable shoots. For clonal propagation of cauliflower, Crisp and Walkey (1974) used pieces of curd which provide numerous meristems capable of reverting back to vegetative buds in cultures and forming leafy shoots. For rhizomatic plants, such as strawberry and Boston fern, runner tips have been commonly used.

The physiological state of the parent plant at the time of explant excision has a definite influence on the response of the buds. Explants from actively growing shoots at the beginning of the growing season generally give best results (Seabrook et al., 1976; Yang, 1977; Anderson 1980a). The seasonal fluctuations in the response of shoot buds may be minimized by maintaining the parent plants under light temperature conditions required for continual vegetative growth, in glasshouse or growth cabinets.

Bulbs, corms, tubers and other organs that require low or high temperatures and/or exposure to specific photoperiod to break dormancy, should be subjected to the required treatment(s) before excising the bud. In *Narcissus* shoot tips taken from bulbs after cold treatment at 11^{o}C for 6-8 weeks grew faster and survived better in vitro than those from untreated bulbs (Seabrook et al., 1976).

For shoot multiplication through adventitious bud formation, with or without callusing, pieces of root, stem, leaf or nucellus are used. In monocots the meristems of leaves and scales are located at a proximal end where they are joined with the basal plate. Leaf-base and scale-base explants should include a small piece of basal plate (Hussay, 1980).

The choice of the explant also contributes a great deal to the differentiation of shoots in callus cultures. In *Eucalyptus citriodora* shoot buds were formed only by those calli which originated from the lignotuber. Calli derived from the stem or root lacked this capacity (Aneja and Atal, 1969), Similarly, in coffee differentiation of shoots and plantlets occurred only in the cultures of segments from orthotrophic shoots (Staritsky, 1970; Monaco et al., 1977).

(ii) Sterilization: Plant tissue culture media being rich in sucrose and other organic nutrients provide a good substrate for the growth of many bacteria and fungi. On reaching the medium these organisms multiply rapidly, overgrow the plant tissue and eventually kill it. In addition these microbes also release into the medium substances that are toxic to the plant tissue. It is, therefore, of utmost importance in any tissue culture work to ensure a complete aseptic environment within the culture vial. Microbiological tests may be required to identify the presence of certain bacterium which remain latent for long periods in standard PTC medium. But adversely affect the rate of multiplication of shoots and subsequently their rooting ability (Anderson 1980a). Standard methods to check the contamination of cultures from various sources are described in standard textbooks (see Wetter in Constable 1982; Bhojwani and Razdan 1983; Bonga and Durzan, 1987).

The problem of microbial contamination of cultures is especially serious with the tissue derived from field-grown material. In such cases it is essential to include stage 0 in the procedure. Debergh and Maene (1981) have reported that 100% cultures of Ficus lyrata initiated from field grown material were lost due to contamination. The problem was solved by growing the stock plants in a greenhouse at 25 °C and 70% related humidity (RH). This procedure is also being used successfully with many other ornamental plants. In the case of hard to root species i.e. difficult to establish in pots the growing branches of the plants may be loosely covered with the polythene bags to protect the newly developing shoots from air-borne contaminants. Alternatively, it may be possible to take small cuttings (2-6 nodes) from the field-grown plants and grow them for short periods in dark solution inside the laboratory. The shoots develop from axillary bud. These cuttings are considerably less contaminated than those taken directly from the field-grown plants.

(iii) Browning of the Medium: A serious problem with the culture of some plant species is the oxidation of the phenolic substances leached out from the cut ends of the explant. It turns the medium dark brown and are toxic to the tissue. Growth inhibition is more severe in species that naturally contain high levels of tannins or other hydroxyphenols. Even in these species, younger tissues are less prone to browning than the older tissues; for example, young leaves of oil palm provided the best explant but here the extent of browning is correlated to the presence of auxin in the medium (Rabechault and Martin, 1976). Whereas Haramaki (1971) could initiate cultures from shoot tips of Gloxinia, but not from the axillary buds as they oxidized too quickly.

A further factor influencing the extent of browning is the time of the year when the explant is collected. In

Hamamelis, the browning intensity was greater in May and less in July and August (Christiansen and Fonnesbech, 1975). Shoot tip explants of apple `Northern Spy´ were less likely to turn brown if collected in spring or summer (Hutchinson, 1982).

Tissue blackening occurs through the action of copper-containing oxidase enzymes such as polyphenoloxidases and tyrosinase (Lerch, 1981) which are released or synthesized and presented with oxidative conditions when tissues are wounded. Substrates for these enzymes vary in different tissues, being commonly tyrosine or o-hydroxyphenols such as chlorogenic acid. Enzymes and substrates are normally retained with different compartments within cells and come together when cells are injured. Phenols have an important natural function in regulating IAA oxidation. The toxicity of phenol is probably mainly due to reversible hydrogen bonding to proteins. Irreparable growth inhibition occurs when phenols are oxidised to highly active quinone compounds which then cyclize, polymerize and/or oxidize proteins to form increasingly melanic compounds. Betacyanins are released when red beet tissues are wounded in culture. These substances are rapidly oxidized and become completely inhibitory (Harms et al. 1983).

Blackening of tissues and the medium can be prevented by one of the several approaches depending on the species:

1. Removing the phenolic compounds
2. Modifying the redox potential

3. Inactivating phenolase enzymes

4. Reducing phenolase activity and substrate availability

Leaching or dispersal: In species where black exudates is the problem only with the original mother explant and not with subsequent subcultures pretreatment of plant material can help in obtaining viable explants. For example, seeds of walnut were soaked in running water for 24 hours to remove phenolis before they were sterilized and germinated in vitro (Rodriguez, 1982). Explants themselves may be left in sterile water for 2-3 hours after isolation before culturing (Cresswell and Nitsch, 1975; Lane, 1978; Vieitez and Vieitez, 1980b).

Growth of explants can be limited by toxic metabolites even in the absence of blackening. If explants do not show any sign of growth after 3-4 weeks, it may be beneficial to transfer them to a fresh medium. Broome and Zimmerman (1978) found this was successful with shoot tips of blackberry where ascorbic acid, citric acid and cysteine hydrochloride were injurious. When the medium used for fig shoot tips turned brown after two days, explants were transferred to fresh medium, and again thereafter at weekly intervals to minimise possible inhibitory effects (Muriithi et al., 1982). In extreme cases, 5-6 transfers at 2-3 day intervals may be necessary until phenolic oxidation ceases.

Explants are sometimes less damaged by browning if cultured initially on a liquid medium in which phenolics can readily diffuse away. Ichihashi and Kako (1977) found that Cattleya shoot tip explants survived better on a stationary liquid medium containing oxidase inhibitors than on a solidified medium of similar composition, while Zepeda and Sagawa (1981) cultured pineapple shoot tips in the first instance in a rotated liquid medium composed of MS salts and 25% coconut milk. Explants may still discolour a liquid medium, those of Sarcanthine orchids turned a liquid medium brown and needed to be subcultured (Intuwong and Sagawa, 1973). Soaking explants in water or pre-treating them in an unsupplemented medium may also remove factors which prevent the growth and proliferation of physiologically mature buds in vitro.

Another possibility, however, is to try to keep the precursors of polyphenolics in the tissue, and this can be achieved by the use·of an isotonic culture medium. The Chardakov method, also referred to as the Russian dye method (e.g. Ting, 1982), can be used to determine the osmotic potential of the primary emplant and to correct osmotic potential of the culture medium (Maene and Debergh, 1986).

3.3 Stage 2: Shoot Multiplication

This is the most crucial stage and it is the rate of propagation which determines the success of the micropropagation system. Broadly,three approaches have been followed to achieve in vitro shoot multiplication viz. i) callusing ii) adventitious bud formation iii) axillary branching.

(i) Through Callusing - The potentiality of the plant cells to multiply indefinitely in cultures and that their totipotent nature premit a more rapid multiplication of several plant types. The differentiation of plants from culture cells may occur by shoot-root formation or somatic embryogenesis. Where applicable, this is often the fastest method of shoot multiplication and has been suggested as a potential method for cloning plant species. However, there are several drawbacks in this method and, as far as possible, it should be avoided in clonal propagation of cultivars. The most serious objection against the use of a callus cultures for cloning is the genetic instability of their cells. In such cultures the cell divisions are so fast that they are not always proceeded by the formation of nuclear/cell membranes and results in polyploidy and aneuploidy. Further, the method is not applicable to many crop plants and wherever applicable, usually initial regeneration ability of the tissue declines with the passage of time. The somatic embryogenesis from the callus cells is considered to be comparatively safer as it is observed that only diploid cells forms the somatic embryos and with the methods developed for encapsulating the somatic embryos with gelly-like substance and it is believed that the artificial seeds hold a lot of promise in the future. The method is further superior as the somatic embryos are bipolar structures with shoot bud on one end and root primodia on the other end. These, once formed, do not need further care as the micropropagated plants. The artificial seeds have been produced in a number of crop plants such as medicago (Janick 1985, Raudenbaugh 1986). For cloning this method has been applied for a number of important plant species such as Citrus spp. (Button and Kochba, 1977, Chaturvedi, 1979), Coffea canephora, (Monaco et al., 1977), C. arabica (Sandahl and Sharp, 1971): Phoenix dactylifera (Tisserat, 1981) and Santalus album (Lakshmi Sita et al, 1979). This has been applied successfully on a commercial scale with oil palm by Unilevers (U.K.) and the micropropagated plants were airlifted to Malaysia for larg-scale plantation. Unfortunately, the results of these trials are not very encouraging as at the time of flowering 60% of the plants failed to produce normal flowers. In this species, however, in addition to somatic embryos, plants also originated from the callus (Bhojwani, personal communication). Therefore, one has to be cautious when using somatic embryogenesis for cloning plants.

(ii) Adventitious bud formation - Buds arising from any place other than leaf axil or the shoot apex are termed adventitious buds. Strictly speaking, the shoots differentiated from a calli should also be treated as adventitious buds. But in this review, we are restricting the use of the term to those buds that arise directly from the plant organ or a piece thereof without an intervening callusing phase.

In nature also a number of plant species produce adventitious buds from different organs such as: root: (*Phlox* certain clones of Apple, *Dalbergia sissoo*, *Rubus* species), bulbs *(Hyacinthus and Gladiolus)* and leaves (*Begonia, Pelargonium, Saintpolia, Streptocarpus* etc.). Broertjes et al, 1968, have listed about 350 species in which the leaves are capable of producing adventitious buds. In fact, in many crop plants propagation through adventitious buds is a standard horticultural practice. In such plants the rate of adventitious bud development can be considerably enhanced under cultural conditions. For example in *Begonia*, buds normally originate only along the ends but in a medium supplemented with BAP the bud formation is so profuse that the entire surface of the cuttings becomes covered with shoot buds (Reuther and Bhandari, 1981). Takayama and Misawa (1982), have described an aseptic method for mass propagation of *Begonia* x *hiemalis* which allows production of over 10^{14} plants in a year from single 7mm x 7mm segment of a young leaf. *Pinguicula moranensis* is also normally propagated from leaf pieces but in cultures the propagation is 15 folds higher than in nature (Adams et al., 1979). Further, in cultures explants are as small as 20-50 mg in weight, are capable of producing adventitious buds which otherwise fails to survive in nature.

Under the influence of appropriate combination of growth regulators adventitious buds can be induced on leaf and stem cuttings of even those species which are normally not propagated vegetatively, eg. *Brassica* species (Kartha et al 1974a) *Chrysanthemum cinerariefolium* (Roest and Bokelmann 1975), *Linum usitasissimum* (Murray et al., 1977) and *Lycopersicon esculenta* (Behki and Lesley, 1976).

The capacity of young fern tissues to regenerate adventitious shoots is very high. Knauss (1976) regenerated plants from the small tissue fragments resulted from macerating the prothallus of several different fern species. This technique can be successfully applied to saprophytic tissue also. Cooke (1979) homogenized cultured plantlets of *Davallia* and *Platycerium* and on plating obtained a large number of new shoots. Presumably these arose adventitiously from very small tissue fragments. Barlass and Skene (1978, 1980a,b) developed a shoot-tip culture method for grapes which involved the production of adventitious buds from the fragmented shoot apex. Excised shoot tips measuring 1 mm in length (with 2-3 leaf primordia) were cut into nearly 20 portions. When planted on a nutrient medium, each shoot-tip fragment formed multiple shoots. The buds developed adventitiously from the swollen leaf bases. The basal portion of the shoot clusters was divided and cultured to produce more shoots. This process, repeated at two week intervals enabled the production of several thousand plants from a single shoot tip in 3-4 months. Although field performance of these plants in terms of trueness-to-type was not reported, all the plants multiplied by this method were normal diploid. This technique of shoot fragment culture has also been used for the micropropagation of *Asclepia rotundifolia* (Tideman and Hawker, 1982).

Many of the monocotyledonous species with specialized storage organs possess a profound capacity to form adventitious buds (Hussey, 1978, 1980). *Hyacinthus* (Hussey, 1975), *Ornithogalum thyrsoides* (Hussey, 1976) are reported to develop multiple adventitious shoots from almost every organ, even those cultured on the basal medium.

Adventitious formation directly from excised organs is less reliable than axiliary bud proliferation but is a more appropriate approach than the callus method for clonal propagation. Adventitious shoots form uniform diploid individuals in cases where propagation through callusing have produced cytologically abnormal plants (Hussey, 1976; Krul and Myerson, 1980). However, adventitious buds are not always true to type. A serious problem may arise when this approach to vegetative propagation is applied to varieties that are genetic chimeras. Adventitious bud formation in these species runs the risk of splitting the chimeras leading to pure-type plants (Bush et al., 1976; Skirvin, 1978; Cassells et al., 1980; Johnson, 1980). For example, some plants derived from adventitious buds induced on the internodal segments of disbudded apple trees differed from the parental clones in their growth habits, fruiting characteristics and pigmentation. This was ascribed to the complex chimeral nature of these clones (Dayton 1969,1970). Additionally, some cultivar of *Chrysanthemum morifolium*

"Indianapolis" are periclinal chimeras. In the cultivar `CF-2 Ind Bronze´ of this species, petals are composed of three genetically different layers (L). Cells of L-I comprising the upper epidermis, have anthocyanin and carotenoid pigments, those of L-II, which includes the mesophyll tissue, are non-pigmented, and the cells of L-III, which refers to the lower epidermis, have only carotenoids (Bush et al., 1976). It has been suggested that while the colour of the flower is determined by L-I and L-III, its shape, size and vigour are controlled by L-II (Steward and Derman, 1970; Bush et al., 1976). Consequently, plants that originated through adventitious bud development from L-I, either in the cultures of intact petal segments, or upper epidermal peel, exhibited quilled petals and abnormally pigmented mesophyll (containing carotenoids). Plants raised directly from shoot-tip culture, on the other hand, displayed typical chimeral constitution. Although the pigmentation of the flower is contributed by L-I and L-III, it seems L-II can influence the expression of this character of L-I, because the pigmentation of L-I plants was not uniform (Bush et al., 1976).

(iii) <u>Enhanced Axillary branching</u> - Each leaf has an axillary bud in its axil which has the potentiality to develop into a shoot. In nature, these buds remain dormant for various periods depending on the growth pattern and environmental conditions. In some species that have strong apical dominance, it is essential to remove or injure the terminal bud in order to stimulate the growth of the sub-terminal axillary buds. This phenomenon of apical dominance is controlled by the interplay of growth regulators. The exogenous application of cytokinin to the axillary buds may overcome the effect of apical dominance and stimulate the lateral buds to grow in the presence of the terminal bud. However, this effect is only temporary, as the lateral shoot ceases to grow as soon as the exogenous supply of cytokinin is exhausted (Sachs and Thimann, 1964).

Conventional vegetative propagation by the stem cuttings utilizes the ability of axillary buds to assume the function of the main shoot in the absence of the terminal bud. However, the number of cuttings that can be taken from a selected plant is extremely limited, because in nature the vegetative growth is periodic and the minimal size of functional cuttings is in the range of 15-30 cms. In contrast much smaller explants (1-2 cm.) are required for micropropagation and the process of shoot multiplication may be continued throughout the year.

The rate of shoot multiplication can be considerably enhanced by growing a nodal segment in a medium containing a suitable cytokinin at an appropriate concentration with or without an auxin. Due to the continuous availability of cytokinin in the medium the shoot formed by the bud, present a priori on the explant (nodal segment, or shoot-tip cutting), develops axillary buds which may directly grow into shoots. This process may be repeated several times and the initial explant may be transformed into a mass of proliferating shoots. There is, however, a limit to which shoot multiplication can proceed in a single passage after which further axillary branching stops. At this stage, if the miniature shoots are exercised and subcultured in a fresh medium of the same composition, multiplication cycle can be repeated indefinitely. In some species, however, it is not possible to induce the axillary buds from the apical dominance by manipulating the hormonal composition of the medium. As a result, the buds present on the initial explant grows into an unbranched shoot. Thus, the rate of shoot multiplication in such cases depends on the number of nodal cuttings that can be exercised from these shoots at the end of each passage. Fortunately, the rates of multiplication achieved by this method are not very low as in Leucaena leucocephala, 6-7 fold multiplication rate was observed over a period of 3 weeks (Dhawan and Bhojwani, 1985). Enhanced shoot development has been applied for a number of crop plants and is theoretically applicable to any species that provides axillary buds and responds to exogenous cytokinin (Hussey 1980). This method may be initially slower (5-10) multiplication every 4-6 weeks) than the other two methods described earlier, but with each passage the number of shoots increases logrithomically and within the year astronomical figures can be achieved. This method of shoot multiplication is becoming increasingly popular for clonal propagation of crop plants because it allows the maintenance of a high degree of genetic uniformity in the plant population. The cells of the apical meristem are uniformly diploid and are less susceptible to genetic changes under cultural conditions.

3.4 Stage III: Rooting of in vitro formed shoots

Somatic embryos are bipolar structures with a preformed radicle and thus develop into a plant directly. In some cases embryos may enter dormancy after attaining maturity and require special treatment for germination (Mullins & Srinivasan, 1976; Kavathekar et al., 1977).

Axillary and adventitious shoots developed in cultures in the presence of cytokinin, generally lack roots. To obtain plantlets, the shoots are transferred to rooting medium that is supplemented with auxin in contrast to shoot multiplication medium which has cytokinin. The number of shoot multiplication cycles after which the rooting exercise should be started is dictated by the number of plants of each genotype to be cloned and the available nursery facilities. In addition, adult material from tree species may require rejuvenation of shoots through a number of in vitro shoot multiplication cycles before satisfactory rooting of the shoots can be achieved.

For rooting treatment, individual shoots measuring about 1 cm in length are exercised and transferred to a root medium. For some plants to economize the process (in terms of labour & space) the entire shoot cluster at the end of a shoot multiplication cycle is transferred to rooting medium.

After rooting, individual plants are separated and transplanted (as in Garlic; Bhojwani 1980; Bhojwani et al., 1982). In some plants high density shoot culture during rooting promoted root formation (Prunus salicina: Rosati et al., 1980). Wounding of the basal end of the shoot is found to improve frequency of rooting as well as number of roots per shoots in cherry (Snir, 1982). Shoots developed in vitro may take 10-15 days to form a reasonably good root system.

The commercial exploitation of micropropagation is limited due to the high cost of test-tube plants. One method of reducing the cost of production is to minimize the aseptic steps which are all labour intensive. Therefore, transferring the shoots directly to non-sterile medium is attempted in many commercial laboratories. Further, in most plants the roots formed in cultures died in soil and it is the new roots that are formed in the soil which take over. Different methods have been tried for in vivo rooting such as :

a) The basal cut end of the shoot is treated in a standard rooting powder or IBA in talc and planted in a potting mix. Regenerated shoots of Rhododendron and Rubus ideaus root equally well in vivo and in vitro (Kyte and Briggs, 1979; Welander, 1985).

b) A non-sterile method for rooting of in vitro multiplied shoots for a number of ornamental plants has been developed

(Debergh and Maene, 1981). In this procedure the shoots are soaked in 2 mg l^{-1} solution of IBA (non-sterile) for ten days and then transferred to the potting mix. During the auxin treatment the rooting is induced and some shoots even developed visible roots. This method is being used with up to 100% success for over 20 cultivars on *Begonia*. x *tuberhybrida* and many other ornamental plants.

c) Another technique for achieving in vivo rooting is to transfer the aseptically raised shoots directly to an artificial potting mix (eg vermiculite, baystrat, oasis, rockwool, etc.) saturated with a 2 mg l^{-1} IBA solution (Debergh and Maene, 1981). By appropriate misting or irrigation, the initially high auxin concentration is gradually lowered due to leaching. This rooting procedure has been used successfully for *Cordyline terminalis*, *Dieffenbachia*, *Dracaena congesta*, *D. deremensis*, D *parevi*, *Ficus sp.*, *Manihot utilissima* and *Spathiphyllum sp*.

However, in vivo rooting is not always successful. For example, attempts to root *in vitro* multiple *Chrysanthemum* shoots *in vivo* resulted in loss of a large number of shoots due to microbial infection (Roest and Bokelmenn, 1975).

3.5 Stage 4: In vitro Nodulation of Micropropagated Plants

Leguminosea especially members of Papilionoidaae and Mimosoideae, constitutes the largest group of plants endowed with the ability to fix atmospheric nitrogen in symbiosis with rhizobia. The first scientific demonstration of this symbiotic relationship came from the work of Hellriegel and Wilfarth (1888). Enormous amount of information has been generated since, but investigations have been mainly confined to economically important herbaceous crop plants. The nodulation studies on tree legumes are comparatively meagre (Allen & Allen, 1981; de Faria et al. 1984).

Tree legumes with inherent capacity to fix atmospheric nitrogen are prime candidates for reclaiming large wasteland existing all over the tropics. It is because, apart from the ability to survive under harsh conditions, they fix atmospheric nitrogen and over a period of time improve the soil status. Since not much work is being done on the selection of Rhizobium strains for different species and different soil types the full potential of leguminous trees for fixing nitrogen is not exploited. Thus, when our aim is to get mixed biomass production applying tissue culture techniques, it is essential to study their microbial associates (e.g. Rhizobium and Mycorrhiza). Also, while introducing an exotic species, it is essential to introduce the associated microflora also.

The importance of nodulation for tree legumes could be explained by taking the of Leucaena leucocephala. An exhaustive survey of Leucaena leucocephala grown in Uttar Pradesh (India) show that exceptionally high yields are obtained only on highly fertile and well-drained soils

(Chaturvedi, 1983). It was also noted that none of the trees planted in these trial fields, whether nutritionally rich or poor, nodulated. In contrast, plants grown in the campus of Indian Agricultural Research Institute, New Delhi had well-formed nodules. Several Rhizobium strains were subsequently isolated from these spontaneously formed nodules.

Nodules of Leucaena leucocephala were first reported by Wright (1903). Although spontaneous nodulation has been reported on plants growing even on barren volcanic soils (Allen & Allen, 1981), not all soil types harbour the required rhizobial strains (McLeod, 1960; Chaturvedi, 1983). A broad spectrum survey (Trinick, 1908) has revealed that Leucaena leucocephala exhibits a high degree of specificity for its partner. Preferential selection of strains for alkaline and acid soils has also been reported (Norris, 1973). In one study, the nodulation pattern has been compared by nine different strains. The study involved following strains: Lcn 1, Lcn 3, Lcn 7, Lcn 8, Lcn 9 (from IARI) K-28-2 and L n 2 (from the Indian Institute of Science, Bangalore) and CB 81 and NGR 8 (obtained from Papua New Guinea). Whereas CB 81 induced maximum nodulation, it was inferior to NGR 8 in the nitrogenase activity and the growth of the plants in nitrogen-free medium. None of the local strains of Rhizobium were as effective as NGR 8.

The main purpose of micropropagating elite genotypes of any plant is to rapidly produce enough plants for multisite trials. Considering the importance of nodulation on the growth of tree legumes, coupled with the specific rhizobial requirements, it is realized that the nodulation of the micropropagated plants with an effective strain before transfer to the soil is beneficial. In fact, our studies with Leucaena leucocephala have shown that all the nodulated plants survived after transfer to soil as against approximately 40% of the non-nodulated plants (Dhawan and Bhojwani, 1987).

The different aseptic manipulations for this study are as follows:

i) Maintaining Rhizobium cultures: Pure cultures of Rhizobium are maintained and multiplied on YEMA medium (Vincent, 1970). Bacterial inoculum is scrapped from the surface of a 3-day-old culture and streaked on to the surface of the freshly-prepared medium. The inoculated tubes are maintained in dark at 35 °C for 3 days and these cultures are used for nodulation experiments. The three-day old rhizobial cultures are stored in the refrigerator and are sub-cultured at monthly intervals.

ii) Nodulation of seedlings : The aseptically raised seedlings with 5-10 mm long root and the cotyledons still inside the seed coat are used for nodulation experiments following Gibson's method (1963) for large-seeded legume. This involves preparing MHB medium in (or any other N_2 free medium) in 200 mm x 25 mm long tubes capped with aluminium foil, secured with a strong rubber band which after autoclaving are placed in a horizontal position so that the agar slant reaches the aluminium foil. Quarter strength liquid media of the same formulation is prepared separately. The foil is swabbed with rectified spirit and a small hole is made near the agar slant with a sterilized needle. The germinated seed is so placed on the foil that its roots penetrate through the hole and lie on the agar slope. Another hole, for watering and ventilation, is made in the cap slightly away from the first hole and with the help of a sterile syringe, liquid medium containing Rhizobium (ca 10^6 bacterial cells/ml of medium) is added. The hole is then sealed with a non- absorbent cotton. The cultures are maintained at 28+2 °C and are illuminated from the top with fluorescent tubes and incandescent lamps. After four weeks the plants are transferred to sterilized sand and irrigated with nitrogen free inorganic medium. After another three months, the plants are evaluated for their morphological characteristics, total biomass production and nitrogenase activity in the nodules. All these are parameters for estimating efficiency of different strains of Rhizobium. The selected efficient strains are then used for nodulation of micropropagated plants.

In studies with Leucaena leucocephala, we found that though the Gibson's tube method is effective in inducing nodulation, results in initial high mortality rate. Therefore, another method was devised which involved transferring the plants to sterilized 250 ml conical flasks containing approximately 180 ml of quartz sand. The plants are saturated with inorganic media containing bacterial suspension and the flasks covered with pre-sterilized aluminium foil and kept in continuous light at 25±2 °C. After 10 days the caps were removed and the flasks shifted to comparatively high light intensity, provided by fluorescent tubes and incandescent lamps. After another three weeks the plants were transferred to large polythene bags containing quartz sand and were transferred to shade in natural conditions. In another two-three weeks, the plants could be transferred to the field.

There is no other parallel study of nodulating micropropagated plants with *Rhizobium*; however, there are two reports of nodulation of micropropagated plants with actinomycetes *Frankia*. These are of *Alnus glutinosa* (Perinet and Lalonde, 1983) and *Elaegnus auguistifolia* (Bertrand and Lalonde, 1985). It has also been observed that nodulation of micropropagated plants with *Frankia* improves the growth of the test-tube plants and ensures their survival, apart from fixing nitrogen in virgin soils.

So far, no emphasis has been placed on this aspect but recently even the commercial companies (Native Plants Inc., U.S.A. and Plantek International (PTE) Ltd., Singapore) are planning to set up units for this study. The most important being association of mycorrhizae and trees.

3.6 Stage 5: Acclimatization and Transfer of Micropropagated Plantlets to the field

The ultimate commercial success of micropropagation depends on the ability to transfer plant cultures on a large scale, at low cost and with high survival rates. Although considerable efforts have been directed towards optimizing conditions for the in vitro stages of micropropagation, scant attention has been paid to the process of acclimatization of micropropagated plants to soil and environment. Consequently, the transplantation stage continues to be a major bottleneck in the commercial micropropagation of many plants (Earle and Langhans, 1975; Broome and Zimmerman, 1978; Conner and Thomas, 1981; Dhawan and Bhojwani, 1987). Evaluation of the morphological anatomical, physiological and biochemical changes during acclimatization is a pre-requisite for the comprehension of this process in order to develop efficient transplantation protocols.

The two basic deficiencies in micropropagated plants which render them vulnerable to transplantation shocks are their heterotrophic mode of nutrition and poor control of water loss.

High sucrose and organic nutrients in the media, often employed for raising cultures, and the poor light conditions seem to limit the photosynthetic capacity of the leafy shoots (Grout and Aston, 1977; Wetzstein and Sommer, 1982; Donnelly and Vidaver, 1984; Dhawan and Bhojwani, 1987), incomplete development of the chloroplast (Wetzstein and Sommer, 1982) and lower net CO_2 uptake (Grout and Aston, 1977; Donnelly and Vidaver, 1984). Although expression of enzymes are known to be influenced by environmental factors (Buchanan, 1980), no comparative studies have been conducted on in vitro grown, acclimatized, and naturally grown plants for the efficiency of the enzymes involved in photosynthesis. Undoubtedly, such studies are of cardinal importance for providing clues to modulation of vital enzymes functions and manipulate environmental factors (humidity, temperature and light) which, in turn, are synonymous with the induction of photoautotrophism in the cultured plants.

The leaves of cultured shoots and plantlets of Leucaena exhibit total lack of starch grains. In micropropagated plants, starch grains appear after the pretransplant hardening stage, supporting other evidence of photosynthesis inefficiency of the plants in cultures (Dhawan and Bhojwani, 1987).

Scant deposition of the protective epicuticular wax on the leaves of the in vitro grown plants has been regarded as one of the most important factors responsible for excessive loss of water, leading to poor transplantation success (Grout and Aston, 1977; Sutter and Langhans, 1979, 1982; Fuchigami et al., 1981; Brainerd and Fuchigami, 1981, 1982; Dhawan and Bhojwani, 1987). The chemical nature of the wax deposited in vitro is also known to differ from that formed under natural conditions. In our studies with Leucaena leucocephala SEM reveal a substantial increase in the amount of epicuticular wax deposited on the leaves following the transfer of plants out of cultures, attaining a density comparable to that of field-grown plants 6-7 wk after transplantation. These observations were in accordance with the study on the rate of water loss from leaves in different stages; the decline in the rate of water loss coinciding with the increase in the amount of wax deposited on the leaves. However, the efficient use of water by the nature-nurtured plants was not matched by the transplanted leucaena even after five months of growth in field conditions. This may perhaps be due to the difference in the chemical nature of wax deposited; hydrophobic wax, typical of in vitro grown leaves (Sutter, 1984) being more abundant on the transplants than the hydrophilic wax predominantly found in the nature-grown plants. Differences in rate of water loss by leaves at different stages of micropropagation, as noted in leucaena, and have also been reported in Malus domestics (Brainerd and Fuchigami, 1981) Prunus insititia (Brainerd et al., 1981 Brassica oleracea (Sutter and Langhans, 1982) and Solanum lacinatum (Conner and Conner, 1984).

4. FACTORS AFFECTING IN VITRO STUDIES OF MICROPROPAGATION

Besides the nature of the explant, the main factors that affect the mode and rate of in vitro propagation are the composition of the medium and physical conditions in which the cultures are grown.

4.1. Media

The plant tissue culture medium comprises of inorganic salts, vitamins, amino acids and sucrose: in other words, the medium is a basal one. Very often this basal medium is supplemented with growth regulators, complex organic nutrients and/or natural plants extracts to meet the growth requirements of specific tissues.

i) Inorganic salts: In general, the tissue culture medium contains 16 essential elements for plant growth. Some media are simpler than others: Knop's medium (Knop, 1985), for instance, contains only four salts. The most important difference among the various media may be the overall salt level. Depending on the concentration of salts the following three categories of media are identified; high salt, e.g. MS (Murashige and Skoog, 1962) intermediate levels e.g. NN (Nitsch and Nitsch, 1969) and low media e.g. WM (White, 1943). Of these, MS and its modification is the most commonly used medium in micropropagation.

Some plants exhibit better survival and multiplication of shoots in media with low level salts. Explants of *Pinguicula moranensis*, for example, do not survive even on half strength Linsmaier and Skoog's medium (Linsmaier and Skoog, 1965), but only when salt concentration is reduced to one fifth of their level in the original medium (Adams, 1979a). Similarly for shoot multiplication in Dionaea muscipula, another carnivorous plant, MS salts have to be reduced to half strength (Parliman et al., 1982b). The desirability of media type, however, is not always dictated by the salt type in the plant's natural environment. The two examples cited above are those of the plants which are native of poor quality and low fertility soils. There are reports of plants which are inhabitants of high fertility soil to perform better in low salt media such as blueberry (Vaccinium corymbosum; Cohen 1980) and Feijoa sellowiana (Bhojwani et al., unpublished).

Low salt media have also proved satisfactory for the rooting of shoots and that are multiplied on complete media. For example, in the root medium for Digitalis lanata (Erdei et al., 1981); Malus domestica (Lane, 1978; Zimmerman & Broome, 1981); Narcissus (Seabrook et al., 1976); Pelargonium hybrids (Cassells et al., 1970); Prunus ceresifera (Garland and Stolz, 1981) and Leucaena Leucocephala (Dhawan & Bhojwani, 1985), the concentration of the salts was reduced to half and for Rosa hybrida (Skirvin and Chu, 1979) it was reduced to a quarter of the level in the shoot multiplication medium.

ii) Carbohydrates: These have two principal functions in a tissue culture medium : a) To provide energy source and b) maintain a minimal osmotic potential around the tissue. Sucrose is the most commonly used carbohydrate in tissue culture. However, for some plants such as *Anthurium scherzerianum* (Pierik and Steegmans, 1976) and *Fragaria* (Boxus and Quoirin, 1974), glucose has been found superior to sucrose.

Sucrose is generally used at a concentration level of 2-3%, which is not always optimum. In *Leucaena leucocephala*, the rate of shoot multiplication at 4% sucrose has been observed to be twice that achieved with the concentration level at 3% (Dhawan and Bhojwani 1985). Further, lowering the concentration of sucrose, to 1.5 results in very poor rates of shoot multiplication in another cultivar of *Leucaena leucocephala* (Goyal et al., 1985). In *Eucalyptus nova-anglica* adventitious buds are formed in media supplemented with 2% sucrose but further growth occurs only if the sucrose concentration is reduced to 0.25%. If the sucrose is maintained at 2% level, callusing occurs on leaves, stems and in extreme cases on the whole shoot. In Lilium, bulblet formation from shoot tip explants is inhibited at high sucrose concentrations (Takayama and Misawa 1979). At 3% level, bulblet formation is observed in 100% of the explants, while at 9% concentration it drops to about 11%

At the rooting stage, when the aim is to induce autotropism, it is desirable to reduce the concentration of sucrose. This has been successfully achieved in *Dianthus* caryophyllus (Roest and Bokelmann, 1981), Pelargonium hybrids (Cassells et al., 1980) and Leucaena leucocephala (Dhawan and Bhojwani, 1985),

(iii) Growth regulators: The few compounds that occur naturally within a plant tissue have a regulatory rather than nutritional function in the growth and development. These compounds are active at very low concentrations and are known as plant growth substances of plant hormones. Synthetic chemicals with similar physiological activities to plant growth substances are termed as **plant growth regulators**. These chemicals are added to plant tissue culture media and depending on the type and concentration we get different morphogenetic responses.

(iv) Other organic compounds: There are several recognized classes of plant growth substances which can be broadly classified into : Auxins, Cytokinins, Gibberellins, Ethylene and Abscisins.

The first two classes of compounds are the most important for regulating growth and differentiation in plants (Skoog and Miller, 1957). It is through the interplay of these two exogenously supplied growth regulators, and their balance with endogenously produced hormones in cultured cells that morphogenetic responses are determined : cytokinin-to-auxin ratios promoting shoot differentiation and multiplication, and higher auxin to cytokinin ratios favouring rooting. The exogenous requirement for these hormones depends on their endogenous levels in the plant, which in turn varies with the plant species, explant and the phase of development.

Auxins : Auxins are incorporated into media to permit the growth of callus cell suspension and to induce morphogenesis (root and shoot induction/ multiplication in conjunction with a cytokinin). The commonly used auxins in tissue culture are:

IAA	Indole acetic acid
IBA	3-indolebutyric acid
NAA	1-naphthylacetic acid
2,4-D	2,4-dichlorophenoxyacetic acid

For callus induction 2,4-D is found to be the most potent auxin. The process of somatic embryogenesis is often initiated in media containing high levels of auxin (especially 2,4-D), but embryos are not formed until the auxin concentration is reduced, or eliminated completely. For micropropagation auxins are sometimes used in conjunction with cytokinin for shoot multiplication but are invariably used for root induction. The most effective auxins for rooting are IAA, IBA and NAA.

Cytokinins: Cytokinins occur naturally, but their compounds analogues can also be manufactured. The first cytokinin to be discovered, Kinetin was isolated in Prof Skoog's laboratory at the University of Wisconsin, USA following experiments to promote the continuing growth of tobacco callus. Other naturally occuring cytokinins are zeatin and

2-ip. The trans-isomer of the zeatin molecule has higher biological activity than the Cis-isomer. Most synthetic preparations of zeatin consist of mixed cis-and trans-isomers.

Despite the natural occurrence of cytokinins in whole plants, isolated and cultured tissues and small organs are unable to sufficiently synthesize of these substances for sustained growth. The high cost of extracting cytokinin from the natural sources has led to the manufacture of synthetic cytokinins, the most common being Benzylamino-purine. In micropropagation kinetin and BAP are most commonly used, followed by 2-ip and zeatin.

Gibberellins · When applied to the whole plants, this compound can influence growth and development in a variety of ways such as increasing length, permitting flowering or inducing fruiting and seed set. Several effects of gibberellins on whole plants are caused by its effect on change availability of endogenous auxins and also due to stimulation of the synthesis and activity of specific enzymes. Plant tissue cultures can generally be induced to grow and differentiate without gibberellins, although gibberellic acid (GA_3) may become an essential ingredient of the media for culturing cells at low densities (Stuart and Stuart, 1971) or where shoot elongation is a problem. Gibberellic acid (GA_3) is the most commonly used gibberellin

Abscisic Acid: Abscisic acid (ABA) is another naturally-occuring growth substance that is synthesized in plastids or chloroplasts. It is a plant growth inhibitor, contrary to cytokinins, auxins and gibberellins. In whole plants ABA is involved in the induction of dormancy but has special regulatory roles such as regulation of stomatal openings. In tissue cultures it is both stimulatory and inhibitory: in some cases, it has been found to stimulate callus growth such as *Cryptomeria* 1 mg/litre of ABA has the same effect as 10 mg/litre of BAP for callusing or adventitious bud formation (Isikawa 1974).

Ethylene: This is produced by living plant tissues and is a growth substance with many regulatory functions. There is often a self-adjusting balance between natural auxin and ethylene levels. Several synthetic ethylene-releasing chemicals have been discovered and the most commonly used in plant tissue culture experiments is Ethephon. However, its use in micropropagation is limited as it is found to be inhibitory. In cases like orange, low concentrations of ethephon (0.01-1.01 mg/litre) have been found to stimulate somatic embryogenesis; on the other hand, higher concentration levels are observed to be inhibitory (Kochba et al., 1978).

Activiated charcoal: Though not a growth regulator, it is frequently added to media as it adsorbs inhibitory substances produced by the tissue; thus, it is able to bring out morphogenesis specially in Gymnosperms, and rooting in hardwoods

The difference between the auxin and the cytokinin for morphogenesis can be represented as follows:

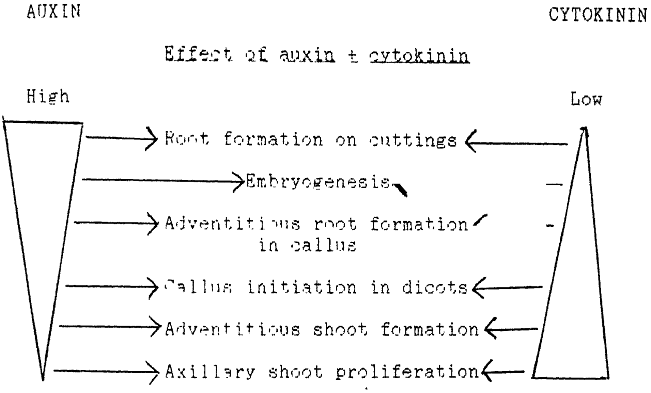
AUXIN
CYTOKININ
Effect of auxin + cytokinin
High
Low
Root formation on cuttings
Embryogenesis
Adventitious root formation in callus
Callus initiation in dicots
Adventitious shoot formation
Axillary shoot proliferation

Fig. 3: The relative amounts of an auxin and a cytokining that are often required to bring about some kinds of morphogenesis (George and Sherrington 1984).

(v) Other organic compounds: Given the essential raw material, green plants are capable of synthesizing all essential amino acids used in protein synthesis. However, the level of amino acids synthesized is sometimes far too low than that is required by the growing and dividing cells in cultures. Therefore, cultured tissues which are exercised organs exhibit better growth in the presence of supplied individual amino acids such as L-glutamine, L-asparagine, L-lysine and Casein hydrolysate, a complex mixture of amino acids (Rangaswamy, 1961). Adenine sulphate, when added to the medium, also sometimes behaves like a cytokinin and enhances growth and shoot formation (Murashige 1974).

Riboflavin (10µ) is reported to improve the quality of the root system in *Eucalyptus ficifolia* (Gorst and de Fossard 1980). In the presence of IBA and under diffused light (10 Em^{-2} S^{-1}) it induces the formation of long roots which grow into medium and bear very short laterals. In contrast, the roots that develop in the absence of riboflavin are short bore laterals, and grow near the surface of the medium. The discovery of Inositol in coconut milk and its growth promoting activities by Pollard et al., (1961) has led to its incorporation as an essential ingredient in the plant tissue culture media.

Nutritive mixtures of composition such as coconut milk, corn milk, malt extract, tomato juice yeast extract, potato extract, etc. are also being used to promote the growth of certain calli and organs (See Bhojwani & Razdan 1983). The use of such undefined substances should, however, be avoided as the response will vary depending on the sources and the time of the year when the fruit is harvested.

(v) *Agar*: In tissue culture studies, both liquid and semi-solid media have been used. However, for the sake of convenience in handling, media jelled with agar (0.6-1%) are generally used. In liquid media, aeration of the tissue in regulating media the ratio of the tissue must be ensured. However, deciding the physical nature of the medium on the basis of sheer convenience may be unwise (Murashige, 1974). For example, the growth of *Cymbidium* explant in agitated liquid media is over 100% greater than in solid media (Wimber, 1965). For *Cattleya*, liquid medium is better at the initiation and multiplication stages while semi-solid medium is satisfactory for rooting (Lindemann et al., 1970). Leaf-tip explants of *Epidendrum* do not survive in the semi-solid medium. However, the calli raised from leaf tips in liquid cultures proliferate well in semi-solid medium (Churchill et al., 1970). Proliferation of *Vanda* explants on semi-solid medium is found to be 8% as against 30% in liquid medium (Kunisaki et al., 1972).

Cultures of most bromeliads (Hosaki and Asahira, 1980) can be initiated only in liquid medium. The establishment of growth of blackberry shoot-tip cultures are also stimulated in liquid medium. Three weeks after the initiation of the cultures, it can be transferred to and multiplied in a semi-solid medium of otherwise identical composition (Broome and Zimmerman, 1978; Zimmerman and Broome, 1980a). Apple shoot-tips cultured in liquid medium for 2-4 days also improved their survival rates (Zimmerman and Broome, 1980c). Similarly, the growth of *Pyrus persica* shoot buds is significantly greater in liquid than in solid medium (Hammerschlag, 1982a).

(vi) p^H of the medium:

Usually the p^H of the medium is adjusted to 5.5-5.8. However, members of the acid-loving heath family

(Ericaceae), such as blueberries and rhododendrons (Anderson, 1975; Kyte and Briggs, 1979) and the carnivorous plants, e.g. *Dionaea* (Parliman et al., 1982a), which grow on poor soils, respond better at lower p^{H} (4.0).

4.2 Physical Factors

i) *Light* : Shoots developing in vitro, although green, are heterotropic, deriving their organic nutrients from the culture medium. The light requirement of most cultures is, thus, only for certain morphogenic processes. Low light intensity in the order of 1,000-5,000 Lux with a 16 h day-length provided by fluorescent tubes is adequate for micropropagation.

In the case of certain plants it may be necessary to maintain the cultures initially in the dark. Calli of *Eucalyptus grandis* initiated in light do not survive due to the oxidation of phenols leached out from the cut ends of the explant. The problem is overcome by keeping the cultures in the dark for seven days from the date of initiation (Cresswell and Nitsch, 1975). Shoot differentiation in the callus cultures of *Allium cepa* can be induced only in the dark and for further growth of the shoots, the cultures are shifted to light (Dunstan and Short, 1979). In members of the family Iridaceae, even the differentiation of shoot buds occurs only when calli grown in the dark are transferred to light (Bajaj and Pierik, 1974; Hussey, 1976c).

Light intensity also affects the type of growth. Increased light intensity (from 3,000-6,000 lux) has been observed to increase shoot formation in one cultivar of *Begonia* x *hiemalis*. High light intensity (1,000-3,000 lux) favoured differentiation of cladophylls of *Asparagus* speares (Hasegawa et al., 1973).

In micropropagation it is customary to maintain the cultures under the same light conditions for rooting as for shoot multiplication. However, light inhibits root initiation in plant like *Santalum accuminatum* and *Santalum lanceolatum* (Barlass et al., 1980), *Malus domestica* (Druart et al., 1982) and *Cotoneaster*, *Crataegus*, *Malus*, *Potentialla*, *Prunus*, *Pyracantha* and *Spiraea* (Norton & Boe, 1982) are seen to exhibit more rapid root initiation in the dark than in light.

Since rooting is also the preparatory stage for transplantation, induction of autotrophism should be kept in mind; for this, it is better if the plants are transferred to comparatively higher light intensity. In fact, in some plants like *Rosa hybrida*, higher light intensity favours rooting (67 $\mu Em^{-2} S^{-1}$ and is higher than that for shoot multiplication stage (17 $\mu Em^{2} S^{-1}$; Bressan et al., 1982).

ii) Temperature

It is customary to incubate the cultures at 25±2 °C; the growth rooms are maintained at the same temperature both during the day and at night. Warm temperatures (about 270°C) are optimum for tropical genera and cool temperatures (about 20°C) for temperate genera (Murashige, 1977). However, many micropropagation laboratories have only a single growth room in which lighting is varied but temperature is fairly uniform. Various plant-types can be successfully multiplied under such conditions, but there are frequent examples in the literature to prove that different species have their own distinctive temperature optima.

The effect of temperature on the cultures of nodal explants from the floral axis of *Phalaenopsis* has been studied (Tanaka and Sakanishi, 1979). Irrespective of the temperature 20°-28° or the buds on the nodal pieces from with nodal bases from termiral portion remained dormant and those on the basal segment formed only vegetative structures. However, in the cultures of nodal pieces from the middle region, the growth response is seen to alter with change in temperature. At 20° and 25°C mostly reproductive structures are formed while at 28°C only vegetative growth occurs. The best plantlet growth from the floral meristems in cauliflower curd tissue occur at 18° and not 22°C (Walkey and Woolfitt, 1970). Leaf blade explants of *Vitis* survive in culture at 20°C; at 25°C buds do not grow. Growth is found satisfactory only at 29°, 32° and 34°C. The explants survive for only a short while (Favre, 1977). Shoot tip explants of peach survive at 80-90% frequency in the temperature range of 21-24°C and only 70 survive at 28°C (Hammerschlag, 1980, 1982a).

Callus cultures also show differences in growth rate corresponding to temperature. Adventitious shoot initiation of *Begonia* x *cleimantha* leaf explants is optimum in leaf cuttings at 15°C (Heide, 1965) and petiole segments at 15-20°C. Further shoot development, however, is enhanced at 24°C. The initiation process is ushered in by cytokinins and in both series of experiments, incubation at higher temperature (25-27°C) inhibits kinetin-induced bud formation. Similar results have been obtained by with leaf explants of Heloniopsis (Kato and Ozawa, 1979). Shoot bud formation here is independent of cytokinin and is optimum at 21-25°C. Treatment at 30°C for seven days or more reduces the number of buds eventually formed in segments of both young etiolated and mature green leaves, but not in segments of young green leaves unless they are kept in the dark at the same time. It has been reported that shoot initiation in *Heloniopsis* (Kato and Ozawa, 1979) is enhanced after maintaining explants at 16°C for 17-21 days before culture at the optimal temperature.

In our studies with *Leucaena* *leucocephala* also, we found that rate of multiplication is affected by temperature. At 15°C, rate of multiplication was 1.7 fold, at 25°C, 2.5 fold, at 30°C, 6.2 fold and at 35°C, 4.8 fold suggesting thereby that 30°C is optimum.

5. TISSUE CULTURE OF FOREST TREES

Natural forests have, to date, been exploited without regard to their management or any efforts being made to develop silvicultural practices for ensuring high quality of plants. A situation has been created whereby traditional reforestration programmes are incapable of keeping abreast of the demand for biomass. This is further complicated by the long life span and multiplicity phases of many forest species; however, methods of countering these problems are now slowly being formulated. Plant tissue culture techniques can play the role of catalysts in achieving this goal. Many of these techniques involve either vegetative propagation or plant improvement through somatic hybridization and genetic engineering. For successful propagation, it is necessary to regenerate entire plants so that they can survive outside the culture vessel, and thus ensure low cost of production

5.1 Historical Perspective

Extensive work has been done on the culture of cambial tissues from a variety of woody species as early as the 1930s (Gautheret, 1934-1940). However, it was not until 40 years later that entire plants could be regenerated from angiosperm (triploid tissues of *Populus tremulides*: Winton 1970) or coniferous material (*Pinus palustris*: Sommer et al., 1975) by organogenesis from callus and adventitious bud development of cotyledons, respectively

In the past decade, there have been numerous reports on the regeneration of complete plantlets from hard as well as soft wood However, the survey of the literature shows that most of this research is restricted to the labs; large scale plantations/field trials are being carried out for a few species only.

For a more detailed review, historical perspective and methodology the following readings are suggested: Karnosky, 1981; Mott, 1981; Brown & Sommer, 1982; David, 1982; Dunstan & Thorpe, 1984; Thorpe & Biondi, 1984; Dhawan & Bhojwani 1986; Dunstan & Thorpe, 1986; Bonga & Durzan, 1987.

5.2 The Importance of Cloning Forest Trees

Trees, unlike agricultural crops, have been difficult to improve genetically because of their long generation times and the prevalence of out-breeding. Although some genetic gains have been achieved by tree-breeding including hybridization, foresters have traditionally improved yield and quality by provenance transfer, as for example in tropical conifers (Gibson et al, 1983) and *Eucalyptus* (Eldridge, 1978). Recently, however, there has been a growing interest in the techniques of clonal forestry and in exploiting the considerable genetic variation present within wild populations. This interest is due to the increasing number of tree species that have been found amenable to vegetative propagation (traditional methods or aseptic techniques), and the realization that clonal selection provides an opportunity to directly harness and exploit genotypic variation. These techniques could also make investment in forestry more attractive by: (i) increasing yields and quality, (ii) shortening rotations, (iii) allowing some of the biological problems hindering reforestation with hardwood species to be circumvented (Leakey et al., 1982; Leakey, 1986).

With regard to the opportunities open to foresters, Libby (1985) has listed the following advantages of clonal forestry:

(i) The ability to rapidly capture a greater proportion of the additive and non-additive genetic variation than can be achieved by breeding, although long-term advances will still depend on breeding;

(ii) The elimination of inbred individuals from production plantations;

(iii) The mass production of valuable but expensive genotypes by hybridization or genetic engineering.

(iv) The mass production of those rare individuals which have two or more favourable characteristics which are usually negatively correlated;

(v) The ability to select and utilize greater genetic diversity than is normally found in a single progeny;

(vi) The ability to use clones that are well adapted to a particular site;

(vii) The greater simplicity and flexibility of managing sets of stockplants as compared to seed orchards;

(viii)The shorter time-span, compared to seed orchards, between selection and production;

(ix) The increasing superiority of clones passing through multiple-trait selection programmes; and

(x) The ability to use maturation states other than the juvenile.

With these advantages, Libby suggests that a decisive shift to cloning forestry is likely, particularly when unit costs are seen to decline.

To date, there are relatively few examples of clonal forestry in the tropics, although a great deal of academic interest has been generated and an increasing number of tree species have been vegetatively propagated. Tropical trees are particularly amenable to clonal forestry as compared to temperate species. Propagation is relatively easy and their rapid growth shortens the selection process. The subsequent pages elucidate the success achieved so far on a case-by-case basis.

5.3 Developments in Clonal Forestry

(i) *Eucalyptus*

Large-scale reforestation programmes with clonal *Eucalyptus* spp. are in progress in the People's Republic of Congo and in Brazil.

Congo: The work by Centre Technique Forestier Tropical(CTFT) started in 1953 with the introduction of 63 species, of which

E. *tereticornis*, E. *urophylla*, E. *cloeziana*, E. *torelliana* and E. *alba* appeared to perform best in the savannahs around Ponte-Noitre. Extensive provenance trials (eg. one with 102 provenances of E. *urophylla*) were then established, in which phenotypic variation among individual trees was found to be considerabe (Delwaulle, 1983). Progenies from these trees included some inter-specific hybrids, which were eventually identified as E. *tereticornis* x E. *saligna* and E. *alba* x E. *urophylla*. The apppearance of these hybrids stimulated the development of techniques of vegetative propagation using leafy stem cuttings (Martin and Quillet, 1974; Chaperon and Quillet, 1978) which are the basis of the clonal *Eucalyptus* programme currently in use in the Congo and Brazil.

Having developed a propagation technique, plus-tree selected within plantations were felled and multiplied using cuttings from coppice shoots, so that clonal trials could be established. The selection programme in Congo had, by 1980, identified 174 superior genotypes of E. *alba* x E. *urophylla* and 256 of E. *tereticornis* x E. *saligna*, using tree height, form, yield, rooting ability and pulping quality as the criteria for selection.

The commercial stage of this programme started in 1978, with the creation of the Industrial Afforestation Unit of the Congo (UAIC) and the establishment of 650 ha of plantations. Some 3000 ha having been actually planted, by the end of 1984, the total area under clonal *Eucalyptas* was about 20,000 ha. Propagation on this industrial scale necessitates some modification and rationalization of the techniques used (Delwaulle et al, 1983). Planting, in this savannah area, is done during the rainy season (December-March). To meet the current annual biomass requirement, about 600,000 cuttings per year are collected from each of the two UAIC propagation units. Each of these units is supplied by about 20 ha of managed stockplants established at a density of 400 per hectare. Cuttings are collected from 95 stockplants per day, with a second harvest 6 weeks later, the total amount to an annual production of about 150-200 cuttings per plant in the season. This process is then repeated every two years. About 12,000 cuttings are set under continuous mist in each nursery each day during the season by two teams each consisting of six people. After 25 days, the misting is progressively decreased, and shading increased until the 40th day, when rooted cuttings are transferred from the propagation area to await planting. Rooting success is usually in excess of 80%.

The clones are planted in November at 5x5 m spacing, in 50 ha monoclonal plots. These plantings are prepared and maintained according to a defined work schedule which ensures both the rapid and successful establishment of the cuttings at minimal cost (Laplace and Quillet, 1983). The uniformity of the clonal material permits both planting at final spacing and the use of mechanical weeding devices. Consequently, in 1980, the cost per hectare was about $650 US, this being less than the establishment cost of seeding. In these plantations, mean annual increments from selected clones after 6 years have averaged 35 m^3 ha^{-1} a^{-1} compared with 20-25 m^3 ha^{-1} a^{-1} from selected provenances and about 12 m^3 ha^{-1} a^{-1} form unselected seed lots.

Currently, a programme of controlled pollination is creating a wider range of hybrids some of which seem to be superior to those already used. Among the most promising are *E. urophylla* x *E. grandis* and *E. alba* x *E. grandis* (Delwaulle, 1983).

Brazil: The rooting of *Eucalyptus* cuttings in Brazil by "Aracruz Florestal" started in 1975, with the objective of clonally producing bleached wood pulp. Within a few years, planting reached an industrial scale. Canker, caused by *Diaporthe cubensis*, was minimized by selection for resistant clones, and improvements of yield, form and coppicing ability were added by further clonal testing (Campinhos and Ikemori, 1978; 1983). Initially, plus-tree selection was carried out in *E. grandis* and *E. urophylla*, and have been cloned, and around 5,000 are currently being tested. The following selection criteria are presently being used: stem volume, based on diameter at breast height and tree height; natural resistance to canker; stem form; natural pruning; thin branches; dense, well-formed crowns to shade out weeds; smooth bark; good coppicing ability; good rooting ability - over 70% success; wood density at breast height - preferably for about 600 kg m^{-3} and high yields of unbleached pulp from

15g samples collected at breast height.

In 1980, the second year of the large-scale planting programme, some 5 million cuttings were planted. If the yields of these match those found in earlier clonal trials three and a half years after planting, mean and annual increments between 45-75 m^3 ha^{-1} a^{-1} can be expected from the hybrid clones planted at 3 x 3. This should be compared with yields from natural seedlings, which here have on an average yielded 36 M^3 ha^{-1} a^{-1}, after 7 years. Campinhos and Ikemori (1983) expect greater gains than these, once silivcultural trials and further selection have proceeded. Refinements of the technique, including some mechanization, are also expected to lower the costs, which are already similar to those of producing seedlings.

(ii) *Gmelina arborea*

A clonal approach to the improvement of *Gmelina arborea* was started in 1981 at Sabah Softwoods near Tawau in Sabah, with the objective of improving stem straightness and branching habit. From a total of 1,200 ha, a 30 ha block of Philippine seed origin was selected as the best stand. The best 100 trees per ha were chosen (a 10% selection intensity) on the basis of tree height, bole length, diameter at breast height, stem straightness, height of first forking, bole circularity and the persistence, size and angle of the branches (Sim and Jones, 1985). These trees were coppiced and, after propagation, 20 ha of stock plants were established at 1x2 m spacing. In 1981, half a million cuttings were planted from the 3000 selected clones. Cuttings are at present harvested at a rate of 20,000 cuttings per day and, when set under mist, have initiated roots in as little as 5 days (See Leakey, 1987).

The results from clonal plantings, and also from seedlings originating from an open-pollinated block of plus-trees were a 100% increase in the number of [illegible] year old-trees classified as 'straight' and a major reduction in those badly forked. The incidence of less severely forked trees were halved. Growth rates (diameter increment at breast height/month) were also improved, particularly in the clonal plantings, which have in addition provided the benefits of allowing planting at wider spacing. A further and more intensive clonal selection programme is planned and greater improvements in form and yield are envisaged.

Large-scale clonal Gmelina plant [illegible] planned in the Solomon Islands and [illegible] in Cameroon ([illegible] 19[illegible]). [illegible] wood quality [illegible] potential [illegible] clonal selection.

(iii) Triplochiton scleroxylon

Nigeria. To overcome the problems of seed availability in Triplochiton scleroxylon (Jones, 1975), experiments to develop vegetative propagation techniques were started in 1971 at the Forestry Research Institute of Nigeria (FRIN) and in 1973 at the Institute of Terrestrial Ecology (ITE) in Britain Methods based on traditional horticultural techniques were soon devised to overcome the previously-reported difficulties in obtaining good root initiation in stem cuttings of this species (Howland 1975 a,b,c; Leakey et al, 1982a) Good rooting requires a propagation environment that minimizes physiological stresses (a simple polythene-covered frame is excellent), an auxin application of about 40 μg of indole-3-butyric acid (IBA) and a leaf area of about 50cm^2, thereby optimizing the balance between photosynthesis and transpiration (Leakey 1983, Leakey and Mohammad, 1985); the most important factors for rooting being (a) stem length in single-node cuttings of more than 30mm, (b) the retention of relatively few shoots per plant, and (c) the interactions between nutrients and the light environment (Leakey, 1985, 1987) These results have been used to develop a sound, cost-effective system of stockplant management, based on physiological understanding of the factors affecting rooting-ability Without this managment it is likely that rooting success, as reflected by the time taken for root initiation, the number of roots per cutting and the percentage of cuttings rooted, will decline with time and with each successive crop cuttings There is also the risk, which must be avoided, that rooted cuttings originating from non-vigorous stockplants will develop into persistently plagiotropic and commercially unacceptable plants The importance of good stockplant management must, therefore, not be overlooked.

The production of clonal material by vegetative propagation of T. scleroxylon seedlings of different origins within the natural range allowed (a) the establishment clonal trials, (b) the assessment of within-clone and within-seed lot variation, and (c) the determination of selection criteria (Howland and Bowen 1977, Howland et al. 1978).

Statistically significant clonal variation in the wide range of parameters was apparent within 18 months, and after 5 years, both basal log volume and stem form varied by eight-fold in a random sample of 100 clones. In this instance, selection of the 33 clones with above average stem volume and stem form would give genetic gains in excess of 30%. This rises to 80% if only the best 10 clones are selected. The equivalent variation between these same clones attributable to seed lot origin was only two fold.

When looking for selection criteria between clones planted at 4.8 m spacing, there were negative relationships after 18 months between branching frequency and both plant height and DBH. These relationships imply that the allocation of dry matter between the tree's main stem and its branches is an important component of yield, probably controlled by the physiological process of apical dominance. Before tesing this hypothesis, a series of experiments in clonal T. scleroxylon investigated the effects of various physiological and environmental factor on apical dominance, as revealed by the patterns of lateral bud activity following decapitation (Ladipo, 1981; Leakey and Longman Lee, Leakey, 1987). On the basis of these experiments, small plants of a range of T. screloxylon clones were decapitated under a standardized regime and the results correlated with branching frequency of the same clones in a 4-year-old plantation (Ladip, 1981, Leakey, 1987). The resulting good relationship between this 'Predictive Test' and field performance indicates that it should now be possible to reliably predict which clones are likely to be high yielding in commercial plantations (Leakey, 1986). It should thus be relatively easy and cheap to screen seedlings every year for those worthy of cloning prior to their introduction into testing and planting programmes.

(iv) *Terminalia superba*

This species has been planted in Congo since 1950 and CTFT has worked on its genetic improvement since 1974. One hundred plus trees and ten double-plus trees have been selected from each of 10 provenances.

From these plus trees, 67 clones have been established in clonal park by grafting adult scions on to seedling rootstocks. Rooting techniques have been developed (Martin and Quillet, 1974) and used to create six propagation parks (two in Mayombe, one at Loudima and three at N'Gouha II) between 1978 and 1982. These contain three successive vegetative generations of rooted cuttings from the original grafts. Rooting ability increased from 11% to 85% during the successive propagations, perhaps owing to rejuvenation or the reinvigoration of the adult scion material (Koyo, 1985). Three generations of clonal tests have also been established between 1978 and 1982, one at Mavombe testing six clones and three at N'Gouha II containing six, eight and nine clones each. Significant differences between mean clone heights and stem circumferences have been recorded after 3 years growth, but not all clones were better than control seedlings. In part, this poor performance may be due to the use of adult scions.

5 3 Identification of Superior Genotypes

In the projects described above, different approaches have been used to select material for propagation. Alternatives range from (a) choosing relatively few, large, mature trees of superior phenotype ("plus trees") and then facing the problem of how to obtain easily propagated or "juvenile" tissues, to (b) testing for superior genotypes from among large numbers of seedlings or cuttings, which although easily propagated, are of virtually unknown genetic potential. For a variety of reasons, one approach may be more appropriate than the other in a given situation/species, as follows.

Phenotypic selection. This approach will almost certainly ensure some genetic gain at relatively little effort and expense, but may fail to identify the best individuals, because, in the wild, survival to maturity is strongly influenced by chance, and large, well-formed trees may already have been removed by loggers. The situation in plantations, and in provenance and progeny trials is probably different, because all plants have a similar opportunity to survive and become established. However, there is some evidence to suggest that the dominant phenotypes are strongly determined by competitive forces between trees (Ford, 1976). Thus, as in the case of T scleroxylon in Ivory Coast selections from plantations should take into account not only the performance of the individual, but also the relative performance of its nearest neighbours. When trees are selected within both natural or man-made stands, the chosen trees are usually felled, or otherwise simulated (Longman et al, 1978), to induce the formation of easily rooted coppice-type shoots from near the base of the trunk. The subsequently derived clones are then re-evaluated in field trials. Attempts in the Congo to initiate rejuvenated clones of *Terminalia superba* by grafting mature scions on to seedling rootstocks have not been very successful.

Selection of superior genotypes from seedling populations

In this approach, a large number of seedlings, preferably from a wide range of good provenances, has to be vegetatively propagated and established in clonal trials. This approach is being taken up in Nigeria with *T Scleroxyon*, primarily because the existing wild population has been subject to heavy dysgenic selection by loggers. It is a laborious and expensive process and and clonal rankings can change with time. However, in relatively fast growing tropical trees, preliminary selections can usually be made after a few years, provided the selection process continues and the selections made are reviewed and verified. Furthermore, new selection criteria, such as wood quality, must be added as the selection process progresses. In the case of *T. scleroxylon*, it is hoped that the Predictive Test' (Ladipo, 1981; Leakey 1986) described above, will greatly simplify and reduce the expenses of the selection process by allowing a nursery-stage screening and the elimination of the least desirable seedlings prior to large-scale vegetative propagation and field testing. This predictive screening should also allow much larger and genetically more diverse batches of seedlings to be assessed for their genetic potential, than can be achieved by field trials alone.

5.4 Apprehensions about Clonal Forestry

Some people have misgivings that clonal forestry will give rise to large, biologically uniform stands that will be vulnerable to pests, diseases or other hazards. This view forms part of a general recognition that, particularly in perennial crops, successful replacement ecosystems must take into account the key role of genetic diversity. The important question, therefore, is whether clonal approaches to forestry overlook this important principle. Fortunately, so far, the clonal forestry projects described are all working with large numbers of clones and are thus not taking undue risks, although some are planting fairly large monoclonal blocks. It is important to remember, furthermore, that tree impovements should be an on-going process and that selection procedures which are reducing the numbers of clones grown commercially at one end, should at the other be continually introducing new genotypes for testing. Additionally, there is no need to further develop techniques of clonal silviculture to make full use of the already listed advantages of clonal forestry, some of which could minimize the risks. For example, the genetic diversity can, in fact, be more effectively maintained in clonal forestry than in more traditional approaches, by deliberately selecting highly productive but unrelated clones, rather than the continuum of related seedligs from a seed stand/seed orchard. The further development of clonal silviculture needs both more thought and experimental testing if the most appropriate system for growing a wide range of clones is to be achieved both to maximize productivity and to minimize risk. In this connection, Libby (1985) has looked at the economics of silvicultural management in a statistcal analysis of the risks of physical and biotic factors. He suggests that -

(a) a mosaic of several unrelated clones in small monoclonal plantations is frequently the best strategy, particularly when, through experience, many of the hazards are known;

(b) mixtures of 7-25 unrelated clones is probably the optimal strategy;

(c) a mixture of large numbers of clones is as safe as a plantation of seedlings, but the genetic gains will now be as high as could be achieved by fewer clones,

(d) the safest situation is likely to be a mixture of a relatively small number of unrelated clones of different species, this option, however, necessitates the selection of well-matched clones of compatible species,

(e) a mixture of 2-3 clones is probably the worst strategy, because of the increased risk of a larger proportion of the plantations suffering damage that will leave unacceptably few survivors, because of this, it is vitally important that short term commercial pressures do not result in the use of very few super clones.

Another fear of clonal forestry is that rooted cuttings may in some way have root systems that are inferior to those of seedlings, and consequently that clonal plantations are more susceptible to wind. Although seedling trees produce a tap root, most tree species subsequently produce a plate of large horizontal roots radiating away from the trunk and many of those forming on bare-root transplants are in fact adventitious (Deans, 1981). The extent to which the tap root and vertical sinkers then develop on a root plate is very variable between species (Jenik, 1978). It is, therefore, wrong to assume that all mature trees grown from seed have a tap root. Rooted cuttings may tend to form a root plate earlier than the seedling, but there is no evidence that these adventitious roots lack the ability to form sinkers. Unfortunately, little is known about the physiological processes affecting the differentiation of vertically and horizontally-oriented roots. So, while accepting that poor wind stability may occur if poorly rooted cuttings are planted, there seems to be no fundamental reason why clonal plants should be inferior in respect of their root system development. The problem can almost certainly be avoided by ensuring that only the easily-rooted plant material is chosen for propagation and that the conditions and techniques used are those which ensure the rapid formation of a radially-arranged, vigorous root system. To a considerable extent, these requirements will be met by good stockplant management (see Leakey, 1983); the use of the most appropriate auxin concentration and cutting leaf area, and the avoidance of physiological stresses during the rooting period (Leakey, 19[illegible]).

The problems of non-erect or plagiotropic plants have also been raised with regard to vegetatively propagated trees. These undesirable plants are the result of using inappropriate shoots as a source of cuttings, and most commonly occur when cuttings are collected from the crowns of large trees. They also appear, even when cuttings originate form young plants, in species where polymorphism exists between lateral and mainstem shoots. This problem can be avoided by efficient stockplant management and the use of only mainstem-type shoots as the source of cuttings; such shoots are usually distinguished from branches by their phyllotaxy and orientation

5 5 Silviculture

An investment in tree improvement, particularly at the clonal level, should imply the existence of the highest standards of silviculture in order to reap the rewards of the genetic potential This is often the case, but there is the problem that relatively little is known about the silviculture of indigenous species plantations and even less of their ecology A large-scale monoculture of these indigenous species obviously carries the risk of creating imbalance in the food chains of the ecosystem which may result, for example, in the explosion of an insect population to pest proportions In West Africa site preparation techniques prior to establishing commercial plantations of *Terminalia ivorensis* [illegible] that remove all the vegetation and [illegible] to the soil, [illegible] the ground vegetation and a number of [illegible] trees Similarly ecologically-sensitive systems are also being used when establishing short-rotation *Cedrela* coppice under standards at Subri Forest Reserve in Ghana ([illegible] and Earle, 1986) In this case galleries of natural forest are also left in the valley bottoms The likely advantages of these approaches, particular where clonal forestry is to be applied, are that species diversity is retained, thereby minimizing the risks of an ecological imbalance. It is likely that the leaching of nutrients is minimized by retention of the fine root mat, which also sustains the mycorrhizal flora. Observations suggest that, due to effects of this, [illegible] tree growth is considerably faster in areas where [illegible] is minimized. In contrast, the damage done at [illegible] is often worsened by the establishment of a highly competitive monoculture of the [illegible] weed Eupatorium odoratum, which suppresses tree growth.

5.6 The Future

The [illegible] propagation techniques being at a stage where they can relatively easily be modified to suit other species and [illegible] future developments are likely to depend on the [illegible] of funding agencies, governments and [illegible] therefore likely to

[illegible]

[illegible] already in plantation

[illegible]

(iii) the start of tree improvement programmes for species that can specifically benefit from (a) the application of clonal techniques to hybrid progenies and (b) the elimination of constraints such as dependence on [illegible] seed supplies (e.g. Dipterocarps like Shorea macrophylla which can now be easily propagated);

(iii) the start of programmes where clonal selection opens up opportunities not readily attained by traditional breeding, for example, the selection of single purpose clones of multi-purpose agro-forestry species. Hence, with the vegetative propagation of species like Prosopis (Leakey & Last 1980, Felker & Clark, 1981) there is the prospect [illegible] certain genotypes for fodder and others for [illegible]

5 7 In vitro culture

In the longer term, production of selected clones is likely to be achieved by in vitro propagation. While the technique is being commercially exploited for ornamentals, it is still at a developing stage for the forestry species (Appendix 1). Really speaking the problems faced by forest tree species are quite different from those faced by ornamental species. The commercially [illegible] plants belong to high value horticultural [illegible] perennial fruit crops [illegible] and [illegible] plants [illegible] The yield from such crops fetches a premium [illegible] and the present techniques of vegetative propagation being labour intensive it is not economically viable.

Category-wise, forest trees fall somewhere between the horticultural crops and broad acreage crops. On the one hand, forest trees are similar to horticultural fruit trees. Being perennial, seed improvement programmes are slow and difficult, on the other, forest trees are similar to broad acreage plants as each tree is low valued at the time of planting (Rs 0.05-0.90 per seedling depending on the species). Further, they are required in large numbers and the returns from such plantations are expected after 5-10 years. However, with the increasing population and per capita energy consumption, we need to increase our biomass production per unit area and cloning technology holds great promise for the future. The techniques can be exploited for a variety of diverse programmes which could perhaps be demonstrated diagrammatically as follows:

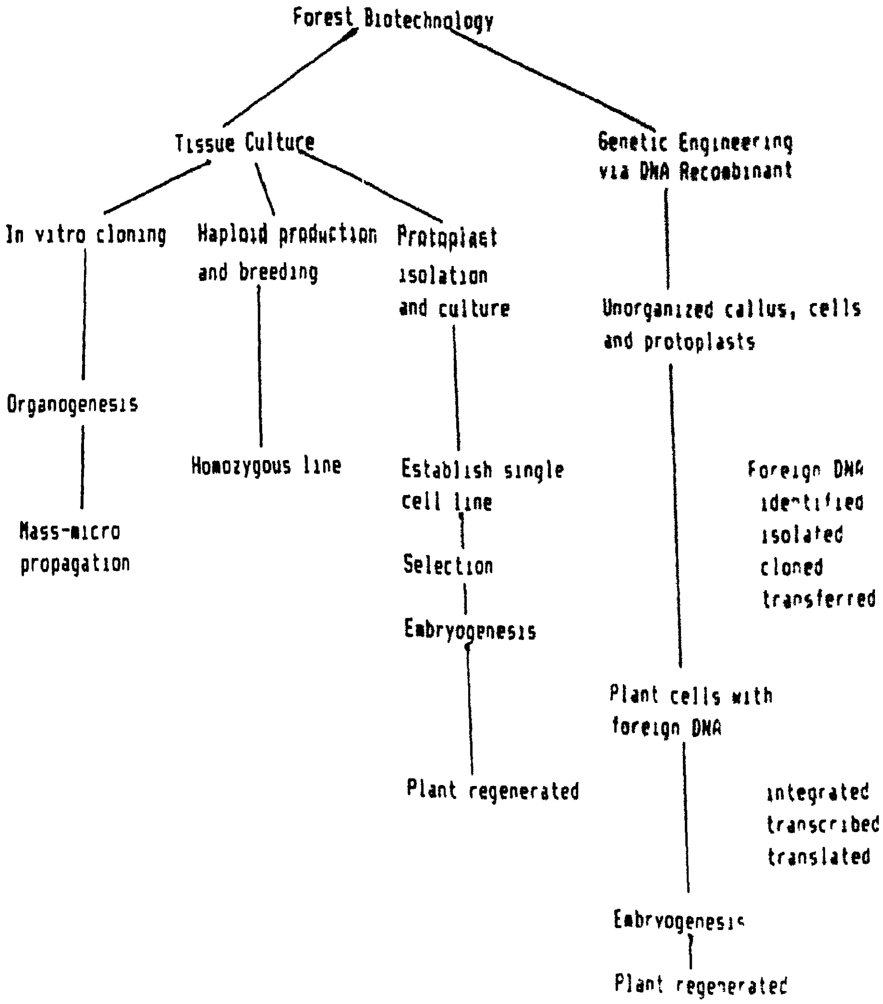
Forest Biotechnology
Tissue Culture
Genetic Engineering via DNA Recombinant
In vitro cloning
Haploid production and breeding
Protoplast isolation and culture
Organogenesis
Mass-micro propagation
Homozygous line
Establish single cell line
Selection
Embryogenesis
Plant regenerated
Unorganized callus, cells and protoplasts
Foreign DNA identified isolated cloned transferred
Plant cells with foreign DNA
integrated transcribed translated
Embryogenesis
Plant regenerated

Fig 3: Forest biotechnology by tissue culture and genetic engineering. On the left, established and developed in vitro techniques; on the right, prospective genetic engineering development. (Source: Chun, 1985)

The problems with tree tissue culture are slightly different from those experienced with other horticultural plants Forest tree species have characteristic juvenile and mature phases that behave differently in culture (as in conventional root-cutting programmes) There is usually a losing ability to vegetatively propagate under standardized conditions as age increases from seedling to the adult tree (Bonga, 1982) Unless and until plant physiologists find a reason for why a tree loses its ability to be propagated or why that trait can be reversed, we will be confronted with the problem of propagating adult trees Further, vegetative propagation is most rewarding using mature individuals and its desirable growth traits can be assessed.

Smith et al , 1982 suggested alternative approach to confront this problem which involves producing sufficient plants from the seedling tissue; evaluate them in the field leading to the selection of clones Since all clones are maintained in cultures, the chosen clone could then be used for mass propagation However, this is just a hypothesis and there is no example of such type of selection and propagation in the literature

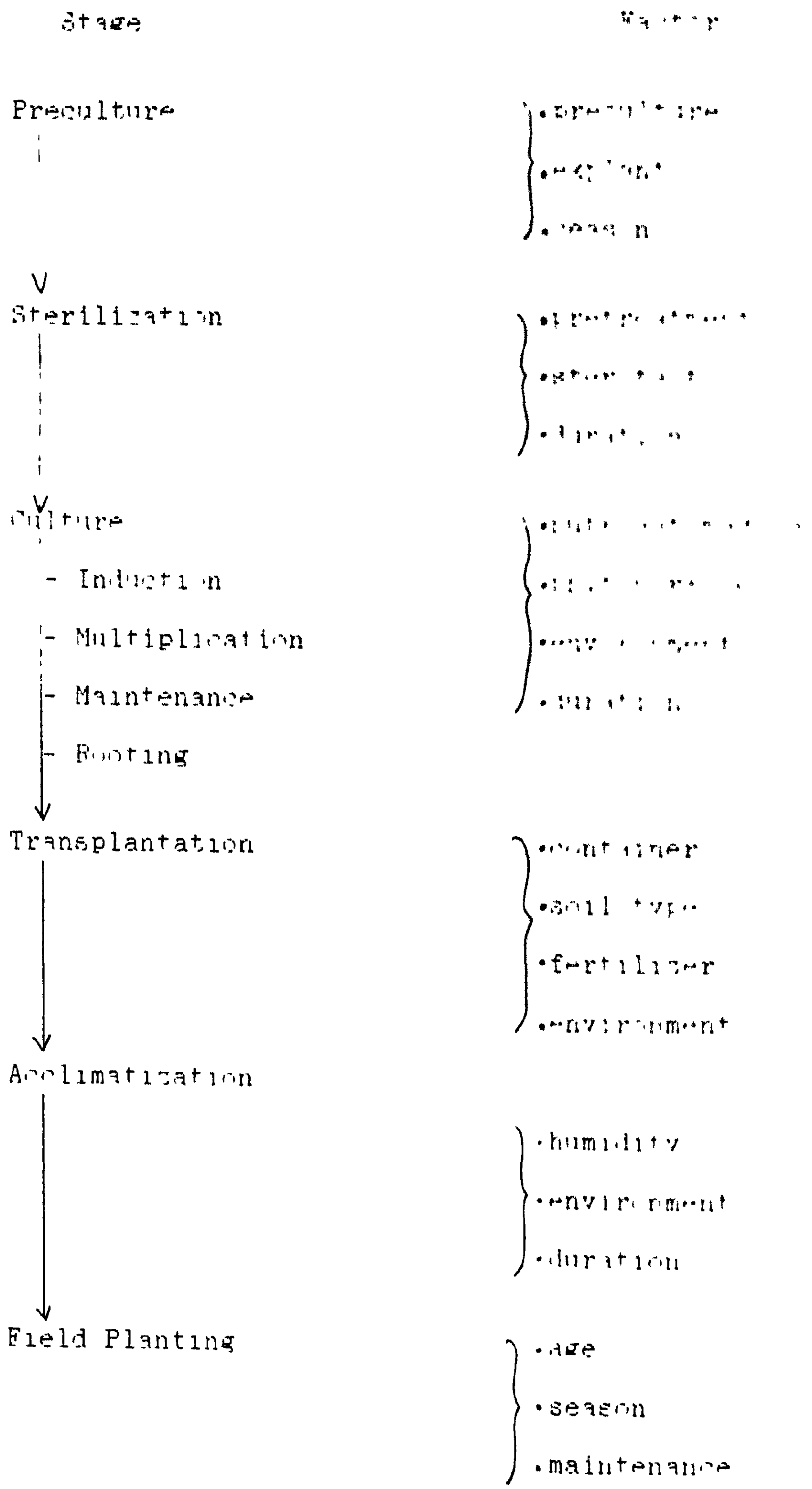

Fig 4 Factors affecting different stages of aseptic cloning of trees (Source : Dunstan & Thorpe, 1986)

Tissue culture to regenerate plants must account for many culture variables (Fig 4) and at present requires an empirical design. One such variable-collection period can be quite critical for forest tree species especially, the conifers. By studying the physiology of growth of the selected species it is often possible to determine suitable supplements or alterations to nutrient media which will permit the extension of the collection period (Dunstan et al 1984). Some aspects of micropropagation especially relevant to tree species are discussed in the subsequent sections.

(i) Explant. Nodal segments are preferred as explants for the micropropagation of adult trees, as excised buds or meristem either do not survive or give poor response. In nodal explant cultures the parent tissues of the mini-cutting seem to contribute significantly towards the survival and growth of the bud. Kyte and Briggs (1979) have described a micropropagation method for Rhododendron in which cultures are initiated from nodal segments and the parent stem tissues are gradually reduced in two or three successive transfers (at two week intervals) before excising the axillary shoots from them. Nodal segments from a four-year-old seedling tree of Feijoa sellowiana exhibited bud break in a large number of cultures. The regenerated shoots could be rooted in vivo with almost 100% success. If, in the process of excising the lateral shoot, their basal node was left intact on the parent cutting, it gave rise to more shoots. However, the excised shoots planted directly on shoot multiplication medium perished after shedding their leaves (Bhojwani et al., unpublished data). The identification of growth promoting factors in the parent stem tissue may help to achieve sustained shoot multiplication in the cultures

(ii) Growth phase of the plant The fact that in vitro propagation of difficult-to-root woody perennials is possible starting from embryonal or young seedlings clearly suggests that micropropagation of elite trees of such species should be possible if they can be induced to develop juvenile shoots by treatments such as suckering, coppicing or hormone application *Eucalyptus* species that have so far been successfully propagated in cultures are those which are from lignotubers These shoots are presumably juvenile

To a certain degree, rejuvenation of plant material can occur in cultures. In some plants shoots formed from the adult material in vitro in the first passage of culture fail to form roots; however, they later acquire the ability to differentiate roots after a number of in vitro shoot multiplication cycles (**McCown** and Amos, 1979, Zimmerman and Broome, 1981) In the cultures initiated from 20-year-old trees of *Eucalyptus citriodora*, the shoots could be rooted only after the third cycle of shoot multiplication Rooting increased from 40% in fourth subculture to 45-50% in the 5th and subsequent passages (Gupta et al., 1981). Similarly, progressive improvement in the rooting ability of mini-cuttings of two difficult-to-root cultivars of Apple was achieved with increasing number of subcultures (Srikandarajan et al., 1982). Freshly cultured shoots did not form roots. In the case of cv. Delicious the percentage of rooting after four and 31 subcultures was 21 and 79, respectively (Srikandarajan et al., 1982)

(iii) Browning of the medium : A serious problem associated with the culture of adult tissues from woody species is the oxidation of phenolic substances that are leached out from the cut surface of the explants (see also Section 3(i)). Phenols occur in plants in a variety of forms and constitute an exceedingly important group of secondary plant products. The oxidation products of phenols are brown to black and are toxic to the living tissues

Where the problem of browning occurs only in the beginning, a pre-treatment of tissues in antitoxidant solutions (Gupta et al., 1980) or culturing them for a few days in liquid medium (Gupta et al., 1981) have been found to be helpful. Shoot-tip explants of *Rhododendron* were soaked in a solution containing 150 $\mathrm{mg\ l^{-1}}$ citric acid or 100m $\mathrm{mg\ l^{-1}}$ ascorbic acid prior to surface sterilization (Anderson, 1975). In *Tectona grandis*, terminal and axillary buds were suspended in a 2% sucrose solution in 100 ml conical flasks and agitated on a rotatory shaker for 45 minutes before planting on a semi-solid medium (Gupta et al., 1980). Keeping the cultures initially in the dark may also reduce the browning problem to some extent (Monaco et al., 1977; Adams et al., 1979)

To overcome the browning when it occurs at each subculture, antioxidants such as ascorbic acid (50-100 mg l^{-1}), citric acid (150 mg l^{-1}), cysteine HCl (100 mg l^{-1}) may be incorporated into the medium (Sondahl and Sharp, 1977, Skirvin and Chu, 1979) Polyvinylpyrrolidone (PVP) has also been used to avoid browning and subsequent death of the cultured tissues (Walkey, 1972, Gupta et al 1980) For *Tectona grandis* insoluble PVP (Polyclar AT) proved superior or soluble PVP With 0 7 % soluble PVP, shoot tips elongated and formed a single shoot while in the presence of insoluble PVP at the same concentration, multiple shoots were formed (Gupta et al , 1980) Incorporation of 0 3% activated charcoal in the medium minimized the problem of browning in the cultures of *Phoenix dactylifera* (Tisserat, 1981)

(iv) *Role of Phloroglucinol* Phloroglucinol (PG) is a phenolic glucoside, a breakdown product of phloridzin The promotory role of PG in *in vitro* shoot multiplication and rooting in woody species is repeatedly emphasized by Jones and his co-workers (Jones, 1976, James and Thurbon, 1979; Jones et al , 1979, Hunter, 1979; Jones and Hopgood, 1979). Shoot tips of apple, cultured on medium supplemented with PG, produced two or three times as many shoots and roots as those cultured in the absence of PG (Jones, 1976, Jones and Hatfield, 1976) Similar effects of PG were observed with

Prunus cerasifera (Garland and Stoltz, 1981), *Prunus institia* (Jones and Hopgood, 1979) and *Rubus idaeus* (James et al., 1980). Even a non-rosaceous tree species has been reported to respond favourably to PG. Shoot-tips of *Cinchona ledgeraina* (Rubiaceae) did not survive more than a week on PG-free medium but continued to grow vigorously for more than a year if the medium contained PG (Hunter, 1979). At one stage it was suggested that the promotory effect of PG was due to the bacteriostatic action of the compound (see Jones, 1979). This hypothesis was, however, proved wrong when Hunter (1979), and Jones and Hopgood (1979), demonstrated that even bacteria-free explants displayed better shoot and root development in the presence of PG. The beneficial effect of PG on shoot multiplication in apple cultivar Northern Spy was observed only in the first two subcultures, by the fourth subculture the promotary effect of PG was lost (Hutchinson, 1982).

Unlike the examples cited above, a large number of workers have found no beneficial effect of PG either for shoot multiplication or for rooting of cuttings (Jones and Hopgood, 1979; Singha, 1980; Zimmerman and Broome 1980c, 1981). Norton and Boe (1982) achieved micropropagation of twelve rosaceous species in medium without phloroglucinol. Working with seven apple cultivars, Zimmerman and Broome (1980) did not observe any significant differences in the rate of shoot multiplication whether or not PG was added to the medium. Subsequently, these authors (Zimmerman and Broome, 1981) reported that PG also did not promote in vitro rooting: for some cultivars it was [illegible] by. Similarly, for the multiplication [illegible] and Hopwood, 1978) and in Tyro, [illegible] PG was found to be without any [illegible].

The only explanation for [illegible] in the response of different [illegible] which there is some [illegible] response to PG [illegible] 1981).

6 COST ANALYSIS OF MICROPROPAGATED PLANTS

Most plant species can be multiplied very rapidly in vitro, if propagation involves a callusing phase. Even in the slowest of micropropagation methods - viz through axillary bud break, astronomical figures can be achieved within a year, as at each passage the number of shoots increases logarithmically A three-fold increase in shoot number every 4 weeks or a six fold increase every 6 weeks, allows more than one million shoots to be theoretically produced from a single nodal segment within a year Some examples of the hypothetical annual rates of multiplication (10,00,000 folds in a year) in the literature are as follows:

Eucalyptus citriodora	Gupta *et al*, 1981
Populus alba	Christie, 1978
Populus nigra	Whitehead & Giles, 1977
Popula tremula	Christie, 1978
Populus flevo (P. deltoides x P. nigra)	Whitehead & Giles, 1977
Salix *matsudana* x alba	Bhojwani, 1980

Such rates of propagation would only be attainable if there was no restriction on the supply of skilled labour for shoot separation and sub-culture, other resources and incubation space Since the subculturing task is to be carried out manually, the production of one million plants within a short time requires a work force of several hundred trained persons.

Apart from the man power, another limiting factor is of incubation space. Although, the shoots can be multiplied and rooted under in vitro conditions throughout the year, their final transfer to the field is restricted to a particular time of the year. To obtain maximum transplantation successes, the plantlets should be of a certain optimal size and this can be achieved only if the multiplied shoots are sent for rooting at certain fixed times of the year so that plantlets of similar optimal size are transferred to the field.

The cost of propagating plants varies from one species to another and according to the technique followed. Usually, in vitro multiplication has a comparatively high cost as compared to conventional methods. Micropropagation is, therefore, only profitable where it has some advantage over traditional methods such as the following

- A plant cannot otherwise be multiplied vegetatively. This is important in the case of dioecious species where seed progeny yields 50% males and 50% females, but plants of one of the sexes are more desirable commercially (such as male plants of Asparagus officinalis and female plants of Carica papaya). Also, in trees, an assessment of desirable attributes can be made when the plant has attained maturity. By this stage, some species lose the capacity to form roots. The seed progeny represents half sibs and is heterogeneous. In vitro clonal propagation would be highly desirable in such cases for the propagation of elite individuals

- As an alternative to the conventional methods of vegetative propagation with the objective of enhanced rate of multiplication This reduces the period between the selection and the release of a new cultivar Also, the amount of plant material released from quarantine being usually limited, tissue culture techniques also hasten the introduction of new crops following their passage through quarantine

- a premium can be attached to providing disease free plants. This is a reliable and economical methods of maintaining pathogen-free plants in a state that can allow rapid multiplication when required.

6 1 Current Economics

Costs related to micropropagation can be identified as the sum of the individual components of production operations and business-related costs incurred, such as direct labour, materials, production overhead in the laboratory, greenhouse operations, sales, and general and administration costs resulting from management of the business.

The economics associated with a labour-intensive production method result in high cost of tissue culture propagated plants. In the U.S., the plantlets from different tissue culture systems are selling for about 30 cents, for which the costs generally break down as 40% labour, 10%

materials, 20% overhead and 30% as sales, general & administrative expenses (Slvis & Walker, 1986) Anderson et al. (1977) estimated that 76% of the cost of producing broccoli plants by tissue culture would be taken up in wage and salary costs (37% for explanting, [illegible] for media preparation and clean up, and 31% for supervision and administration) With such a high proportion of the total related to wages, the actual cost of tissue cultured plants will necessarily differ between countries. For instance, those produced in the U.S.A cost somewhat more in international currency units than those produced in Europe. Goh (1982) mentioned that at that time the price of mericloned orchid plant raised in Singapore was four times greater than that of plants produced by the same technique in Thailand

Another significant component that contributes towards the cost of the micropropagated plants is the overhead expense related to supervision, research & facilities and utilities. Tissue culture laboratories are also energy intensive The utility consumption rates for lights, air conditioning and autoclaving can be significant The capital equipment components of a tissue culture facility contribute to overhead both as variable costs, in terms of utilities consumption, and as fixed costs, in terms of capitalization, depreciation and facilities improvement A typical facility for 5,00,000 plants per year in U S may have initial capital equipment and facilities expenses of $ 250,000 and utilities requirements of $ 500 per month. In India, however, the cost is much higher since most equipments are imported and one has to pay 170% import duty.

6 2 Cost Savings -

The greatest proportion of the cost of micropropagation is associated with the later stages when a large number of manipulations are necessary Costs at shoot multiplication and rooting stages, can be reduced by transferring shoot clumps, rather than individual shoots. The eventual separation of shoots at stage III inevitably takes time and labour Debergh and Maene (1981) reported that tissue culture techniques for cloning being expensive, they could be applied only to limited number of species However, introduction of some additional new techniques may possibly reduce the cost and broaden the range of plants that can be propagated economically in vitro They suggested that stock plants are grown under controlled conditions prior to in vitro culture in order to obtain healthier explants and uniform response (stage 0) and rooting the plants under non-sterile conditions By growing plants at 25^{o} C and RH of 70% for 2 years, 100% healthy cultures of *Ficus lyrata* were obtained in contrast with failure to obtain a healthy culture

For some plants it has been possible to treat the shoots formed in cultures as mini-cuttings and to root them *in vitro* The basal cut end of the shoot is treated in a standard rooting powder or IBA in talc and planted in a potting mix. Regenerated shoots of *Rhododendron* and *Rubus ideaus* root equally well *in vivo* and *in vitro* (Kyte and Briggs, 1979; Welander, 1985); however, in *Vaccinium corymbosum* (Cohen and Elliott, 1979) and *Feijoa sellowiana* (Bhojwani et al. unpublished data) rooting is far better under *in vivo* conditions. Other advantages of rooting the shoots under non sterile conditions are as follows (see Debergh and Maene, 1981): (a) *in vitro* rooting which involves transferring individual shoots to a rooting medium is a labour intensive process. In developed countries it accounts for about 35% of the toal cost of micropropagation process (Debergh and Maene, 1981), with lily it is as high as 75% of the total cost of production (Anderson and Meagher, 1978), (b) usually a delay in growth is manifested for the *in vitro* rooted shoots after transfer to potting mix, and (c) the roots formed *in vitro* may be damaged during transplantation, which considerably enhances the chances of root and stem diseases, eventually, leading to poor transplantation success.

Debergh and Maene (1981) developed a non-sterile method for rooting *in vitro* multiplied shoots. In this procedure, individual shoots are soaked in 2 mg l^{-1} solution of IBA (non sterile) for 10 days and then transferred to the potting mix. During the auxin treatment rooting is induced and some shoots even develop visible roots. This method is being used with upto 100% success for over 20 cultivars of *Begonia* x *tuberhybrida* and many other ornamental plants.

Another approach to achieve *in vivo* rooting is to transfer the aseptically raised shoots directly to an artificial potting mix (e g. vermiculite, baystrat, oasis, rockwool) saturated with a 2 mg l^{-1} IBA solution (Debergh and Maene, 1981). By appropriate misting or irrigation, the initially high auxin concentration is gradually lowered due to leaching This rooting procedure has been used successfully for *Cordyline terminalis*, *Dieffenbachia*, *Dracaena congesta*, *D. deremensis*, *D. parevi*, *Ficus* sp , *Manihot utilissima* and *Spatiphyllum* sp

However, *in vivo* is not always successful For example, attempts to root *in vitro* multiplied *Chrysanthemum* shoots *in vivo* resulted in the loss of a large number of shoots due to microbial infection (Roest and Bokelmann, 1975).

6.3 Market structure

The market size for micropropagated plants is subject to some debate There has been a steady rise in the total number of plants produced annually from tissue culture (both in terms of number of species and plants of a particular species). This is especially true in the case of ornamentals where a new variety or morphotype appears for which either alternate propagation methods are not available or are equally expensive. In case of crop plants for potato, tissue culture techniques are exploited. The crop is planted as a vegetative unit, known as seed piece or tuber section. The potato crop is plagued by a large number of pests which can be spread through seed; therefore, seed piece quality standards have been set by certification agencies. This has now been overtaken by tissue culture to mass-produce early stock of potato seed. The field is currently expanding through the addition of a greenhouse tuber production phase, as a means of providing a grower with a field plantable unit which does not require special handling.

On a commercial scale plant tissue culture is being most widely used for the clonal propagation of plants, popularly called 'micropropagation' Over 250 commercial labs are existing in U.S A and Canada alone, of which around 10 are producing over 5 million plants a year The market for tissue culture propagated plants is largely determined by price Since the process is intensive, the test tube plants are around 10 times more expensive than the seed raised plants It is apparent that the market for plants from tissue culture could expand dramatically if the prices were reduced, the growth of the industry will be largely dependent on technological advancements, especially in the field of automation and mechanization.

While lack of automation is generally regarded as the bottleneck to advancement, progress in this area has been slow It is basically because of the complex physical arrangement of nodes and shoots on a microplant and thus the difficulties in mechanical access to the material being separated. For example a cluster of shoots or individual nodes on stem represents an interconnected framework of tissues and being living systems, shows lots of variability in the configuration of the units e.g the distance between buds or shoots and their spatial orientations are not consistent and accurately predictable in a tissue culture environment Another impediment to automation of tissue culture systems is the shape of the culture vial. They tend to be small, to limit contamination and are normally deep which do not provide easy mechanical access to the shoots being separated.

6.4 Economic Analysis of Use of Tissue Culture in Forestry

The cost of mass producing seedlings for production forestry by tissue culture technique is of much concern to researchers and foresters The current costs of producing tissue culture propagated plants are at least 2 to 3 fold greater than present nursery production costs but the economics depend on the final value of the product In some conifers, detailed studies on the production cost are made One such example is in pines where it is estimated that improved seeds from seed orchard of pine costs $ 1000 00 per pound A pound of seeds results in 20,000 plants and thus seed cost alone comes to $ 50 00 per thousand. An additional expenditure of $ 50 00 is incurred to raise seedlings in the nursery. For a similar product by tissue culture the cost comes to $ 123 per thousand In such cases tissue culture techniques becomes less formidable but with added advantage of large unlimited number of plants produced

Among hardwoods, very few studies have been carried out and unfortunately even those are limited to the research laboratories (Table 1 & 2) But none have been pursued for long i e. to compare the returns from tissue culture plantations vrs seed raised plantations The initial investment for raising plantations from micropropagated plants is comparatively high but one can expect higher returns because of superior uniform clone.

Table 1: Estimated Direct Production Costs per Thousand Containerized Seedlings using Shoot Tip Culture Techniques (1981 U.S. Dollars)[a]

Wages and Salary Costs	$/1000
Explanting Labor	$ [illegible].00
Supervision/administration	$ 5.00
Media Cost and Preparation	$ 8.30
Glassware and Expendible	$ .60
Containers and Nutrients	$ 5.00
Overhead Costs	
Heating, cooling, lights, water, etc	$ 5.00
Total Direct Cost	$ 1[illegible]2.90

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[a] Does not include amortization of facilities and equipment. These estimates are based on information and records maintained in a research rather than an operational commercial facility. Data is based on a 5 fold increase in shoot propagules during a 10-12 week culture cycle using easy to root species such as *Acer rubrum* or *Robinia pseudoacacia*.

Table 2 Comparison of Direct Production Costs per Thousand Plantable Sweetgum Seedlings using Three Different Systems of Production (1980 U S Dollars)

System	Approximate Costs at Production Site
Bare root, 1-0 Nursery Stock From Unimproved Seed	$ 65.00[a]
Containerized Seedlings Using Unimproved Seed	$ 75.00[b]
Containerized Seedlings From Shoot Tip Cultures	$ 123.00[c]

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[a] Personal communication from an industrial pulp and paper company in Georgia. Prices of Sweetgum seedlings proudced by State Forestry Organizations in the SE, USA are commonly sold to landowners below actual costs and ranged from $28.50 to $50.00/thousand (Forest Resources Mgt Tech. Note #4E Cooperative Ext Service, Mississippi State University 1980

[b] From estimates of Vyse and Ketcheson and Mann including 12% annual adjustment for inflation since publication of papers in 1974 and 1977, repectively

[c] Estimated costs by Brown and Sommer for Shoot Tip Cultures of Several hardwood species in a research laboratory not designed for commercial production.

7 PRACTICAL APPLICATIONS OF MICROPROPOGATION

During the last decade micropropagation has become increasingly popular with commercial growers and nurserymen as an efficient method for cloning ornamental plants. For orchids the technique has been in use for an even longer period and, as mentioned earlier, this is the only commercially feasible method for their clonal propagation.

Micropropagation holds special significance in situations where the stock material is extremely limited and requires rapid bulking up. For example, most countries have a stringent and time consuming quarantine system. During its passage through quarantine a large proportion of the plant material is either lost or discarded and only very little material is finally released to the importer. In order to establish a reasonable size stock from the material in hand, conventional methods may take several years. This period can be significantly reduced by efficient micropropagation techniques. Many countries permit direct importation (without going through quarantine) of plant materials in aseptic cultures that can be used as stock for in vitro multiplication. Rapid increase of plant material is also required in breeding programmes where generally, there is just one plant with a set of desired characters. Micropropagation seems ideal for safe and rapid multiplication of such valuable genotypes.

In the cases cited above, micropropagation is required only in the initial stages of `explosive multiplication' to build up a few thousand plants after which the conventional and semiconventional techniques prove unnecessarily expensive. However, for some ornamental annuals and many perennial woody cultivars, where plants or cut flowers are in demand almost throughout the year, tissue culture methods are found profitable for their continual propagation.

In vitro methods of plant multiplication may have permanent advantage over the non-sterile methods of propagation in cases where there are serious problems with diseases. They provide reliable and economical methods of maintaining pathogen-free plants in a state than can allow rapid multiplication when needed, irrespective of the time of the year. Tamarillos (Cyphomendra *betaceae*) can be easily grown from cuttings but is nearly always grown by seeds to ensure that the plant has an upright growth habit and is virus free. In vitro propagation from shoot-tips of adult tree confers the same upright growth habit together with freedom from virus, with the added advantage of clonal propagation of the outstanding individual plants (Cohen, personal communication).

Vegetative propagation is extremely important in dioecious species where seed progeny yields both and male and female plants, but plants of any one of the sexes are more desirable commercially. For example, in *Asparagus* *officinalis* male plants [illegible] female plants but their [illegible] developed [illegible] this [illegible] valuable (e.g., [illegible] comparatively large [illegible] their seed progeny [illegible] plant that [illegible] in terms of [illegible] is to clonally [illegible] plants. In vitro propagation [illegible] reported by a number of workers (Y[illegible] and [illegible], [illegible] and Conover, 197[illegible], **Pandey, 1986;** Litz, 1986), but there is no information on whether or not it is being used on commercial scale.

Some recent reports have suggested that plants [illegible] by micropropagation do not always [illegible] the physiological age of the stock tree. Two [illegible] plantlets of apple cv. Fuji raised by micropropagation technique flowered 10 months after transplantation (Ishihara and Katano, 1982). Similarly, plants of pomegranate raised by tissue culture set fruits within eight months after transfer to the field, under normal conditions it requires more than a year (Mascarenhas et al., 1982).

8. LIMITATIONS OF MICROPROPAGATION

In vitro clonal propagation is being practised on a commercial scale for a wide range of herbaceous and woody species The technique is often in direct competition with the conventional methods, as in *Lilium*, *Ficus*, *Gerbera* and *Prunus* root stocks Independence from seasonal supply, the propagation of plants for which there is no alternative method, freedom from disease and clonal uniformity - all these factors combine to increase the value of this technique beyond its conventional competitors However, there are several problems to be solved before full potential of this technique can be exploited.

The tree species which are difficult to propagate by conventional methods have also proved problematical for in vitro propagation Tissue and cell cultures of several important crop plants do not express their cellular totipotency in cultures These problems should not be insurmountable. Appreciative of the economic importance of these plants, several laboratories are studying methods of inducing controlled organogenesis in tissue culture of cereals, legumes and fibre crops, and to propagate elite tree species

The shoot multiplication method involving a callus phase is often the most rapid method of vegetative propagation; however, the cultured cells are generally genetically unstable. Methods to control these abnormalities could significantly [illegible] in the area of [illegible] their potentiality [illegible] and there is [illegible] phenomenon [illegible] formation with [illegible] *tazetta* (Steinitz and [illegible] x *hiemalis* [illegible] system [illegible] vitro plantlet [illegible]

Time and again [illegible] repeated cycles of in vitro [illegible] cultures show the appearance of water [illegible] ent leaves. Shoots with such leaves suffer [illegible] with and rate of multiplication and may eventually [illegible]. Debergh et al (1981) reported this [illegible] globe artichoke (*Cynara [illegible]*) [illegible] 'vitrification'. Later, [illegible] appropriately as hyperhydric [illegible] and Maene, 1982). In [illegible] increasing the agar concentration [illegible] to remove symptoms of hyperhydric transformation but it also drastically reduced the rate of propagation (Debergh et al., 1981). The hyperhydric transformated shoots formed in shoot-tip culture of carnation rarely survived upon transplantation (Ziv et al., 1981). Decreasing the water potential of the medium by increasing sucrose/agar concentration reduced the vitrification but concomitantly it also adversely affected the rate of shoot multiplication. Culturing the shoot-tips (0.2 0.3 mm) on paper bridges in liquid medium for 5-6 days prior to transplantation on high agar medium (1.5%) allowed a faster rate of growth and multiplication of shoots as compared to those cultured directly on high agar media. To increase the survival frequencies further, the culture tubes were kept for [illegible] weeks in a desiccator under high light intensities prior to transplantation (Ziv et al , 1981)

Once a micropropagation method has been developed and aseptic cultures established it may be tempting to continue to propagate from this stock material for many generations without referring back to the parent plant. This may lead to bulking up of any off types (sports or accidental changes) that might have arisen in the cultures during the early stages. This problem could be serious with perennial tree species where several years lapse before any evaluation of the [illegible] features of individual plants can be made To overcome this problem it is advisable to raise only a few thousand plants from a single explant Many commercial laboratories do not like to go beyond the fourth cycle of shoot multiplication (Zunwalt, 1976)

J CONCLUSIONS

In this review an attempt has been made [illegible] the techniques, applications and limitations [illegible]. This in vitro technique for [illegible] plant genotypes is becoming increasingly [illegible] nurserymen. The number of [illegible] laboratories is steadily [illegible] countries. Micropropag[illegible] aesthetic value [illegible] and virus [illegible]. However, [illegible] of species and cultivars, which [illegible] necessitate further research and [illegible] specific genotypes.

In adopting tissue culture techniques for propagating plants at commercial scale it is of utmost importance that the advantages of the method are carefully weighed against the cost involved. Investment in a tissue culture set up facility is very high. Developing propagation methods for each species is also a costly affair. There is no single medium formula applicable to all plant types. To develop methods for a new cultivar, one has to proceed systematically. The method being empirical, the rate of success cannot be predicted before the work commences. However there are several crop species for which published methods of micropropagation are available (see Appendix 1) that may be adopted with slight modifications. Unfortunately, most of the research, especially on forestry species is limited to laboratories and one must make all efforts to take it to the field and make the technique commercially viable

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For all other references refer to: Dhawan and Bhojwani (1986); George and Sherrington (1984) & Leakey (1987) which are the main sources of information included in this note.

APPENDIX I

Crop plants propagatable in vitro

I(1)Ornamental crops

Plant	Age	Explant	Mode of propagation	Rate of multiplication	[illegible]	[illegible] (%)	[illegible]
1	2	3	4			7	
Acalypha wilkesiana	A	AB	AxB	+	+	100	[illegible]
Aechmea fasciata	A	AB	AdB	4 fold in 12 wk		+	Hosoki and Asahira 1980a
	-	LS	AdB in vitro	6 fold in 4 wk	+	+	Hosoki and Asahira 1980b
Alnus glutinosa	S	AB	AxB	+	+	+	Hosler et al 1981
Alstroemeria hybrid	A	NS, SS	AdB	8 fold in 6-8 wk	+	-	Ziv et al 1973
Amaryllis hybrid	A	SS, peduncle anther	C	+	40	+	bapat and Narayanaswamy 1976
Anemone coronaria	A	ST, peduncle segment	AdB	1,700 fold in [illegible] wk	+	+	Sutter and Langhans 1978
Anigozanthos fuliginosa	A	Rhizome segment	+	2 7 fold in 3 wk	40-100	80-95	McComb and Newton 1981
Anigozanthos manglesii	A	Rhizome segment	+	3 fold in 3 wk	40-100	80-95	McComb and Newton 1981

1	2	3	4	5	6	7	8
Anigozanthos pulcherrima	A	AB	AxB	+	+	+	Ellyard 1978
	A	Rhizome segment	+	4 fold in 3 wk	40-100	80-95	McComb and Newton 1981
Anigozanthos manglesii X flavidus	A	AB	AxB	+	+	+	Ellyard 1976
	A	Rhizome segment	+	4 fold in 3 wk	40-100	60-95	McComb and Newton 1981
nigozanthos preissii X flavidus	A	AB	AxB	+	+	+	Ellyard 1976
nthurium ndraenum	A	Young soft tissue	[illegible]	+	+	+	Pierik et al 1974
	A	AB	AxB	+	+	+	Kunisaki 1980
nthurium cherzerianum	A	Leaf	[illegible]	+	+	+	Pierik and Steegmans 1976
	A	Spadix fragments	[illegible]	+			Geier [illegible]
ntirrhinum majus	A	INS	[illegible]	+	+		[illegible]
sparagus plumosus	NM	ST	[illegible]	+	+		[illegible]
egonia X cheimantha	A	PS	[illegible]	+	+	+	[illegible]
	A	Peduncle Segment	[illegible]	65 fold in 10 wk		-	Appelgren 1976
egonia X hiemalis	A	PS	AdB	+	+	+	Welander 1977

1	2	3	4	5	6	7	8
	A	Peduncle Segment	AdB	85 fold in 10 wk	+	-	Appelgren 1976
Begonia X hiemalis	A	PS	AdB	+	+	+	Welander 1[illegible]
	A	PS	AdB,C	135 fold in 4 [illegible]	+	100	[illegible] and [illegible] 19[illegible]
	A	PS,LS	AdB	150-200 [illegible]	[illegible]	+	[illegible] and Misawa 1[illegible]
	A	PS	AdB	25.9 fold in [illegible]		7[illegible]	Simmonds and We[illegible] 19[illegible]
Begonia tuberhybrida X grandiflora	A	AB	AxB AdB	10 fold in 4 wk	100	100	Debergh (pers comm)
Begonia tuberhybrida X multiflora	A	AB	AxB AdB	10 fold in 4 wk	10	100	Debergh (pers comm)
Betula papyrifera	S	ST	C	15-30 fold in 6 wk	-	-	McCown 1979
Betula pendula	A	INS	C	+	[illegible]	+	Huhtinen and Yohyaoglu 1974
	A	Anther	C	+	+	+	Huhtinen 1978
Betula platyphylla	S	ST	C	15-30 fold in 1 wk	-	-	McCown 1979
Betula schezuanica	S	ST	C	15-30 fold in 4 wk	-	-	McCown 1979
Bougainvillea glabra	A	ST	C	10 fold in 4 wk	60	+	Chaturvedi et al 1978
	A	ST	AdB	100,000 fold in 52 wk	100	+	Sharma et al 198

1	2	3	4	5	6	7	8
aladium sp.	A	LS	?	+		+	[illegible]
ephalotus follicularis	A	ST	AxB	5-10 fold in 1[illegible] wk	+	+	Adams et al 1979
haenomeles japonica	A	ST	AxB	7 fold in 4 wk	+	NM	Norton and Boe 1982
hrysanthemum morifolium	A	ST	AxB AdB	9 x 10 fold in [illegible]	+	+	Earle and Langhans 1974 a, b
	A	Peduncle segment	AdB	+	+	+	Roest and Bokelmann 1975
ordyline stricta	A	NS	AxB	+	+	+	Debergh 1976
ordyline terminalis	A	ST NS	AxB AdB	+	+	++	Kunisaki 1975
	A	ST	AxB	500,000 fold [illegible]	+	+	Miller and Murashige 1976
otoneaster dammeri	A	ST	AxB	7 fold in [illegible]		NM	[illegible]
rassula argentea	A	Leaf	AdB	+	+	-	[illegible]
rataegus rachyacantha	A	ST	AxB	10 fold in [illegible]	+	NM	[illegible]
rataegus X ordenensis cv. 'Toba'	A	ST	AxB	5 fold in 4 wk	+	NM	[illegible] 1982
ryptanthes sp.	A	AxB	AxB AdB	50 fold in 15 wk	100	100	Debergh (pers comm.)

1	2	3	4	5	6	7	8
rataegus X ordenensis cv. 'Toba'	A	ST	AxB	5 fold in 4 wk	+	NM	Norton ad Boe 1982
ryptanthes sp.	A	AxB	AxB AdB	50 fold in 13 wk	100	100	Debergh (pers. comm.)
yclamen persicum	A	TD	AdB	+	+	+	Geier 1977
	A	Anther, LS	C	+	+	+	Geier 1977
ynara scolymus	A	ST	AxB	4-5 fold in 3 wk	84	+	Ancoraet al. 1981
	A	Am	AxB	3.5 fold in 4 wk	85	85	Debergh (pers comm)
aphne X burkwoodii	A	ST	AxB	5 fold in 4 wk	70	90	Cohen and Le Gal 1976
aphne odora	A	ST	AxB	+	+	+	Cohen and Le Gal
ianthus aryophyllus	A	ST	AxB	60 fold in 6 wk	+	75	Earle and Langhans 1975
	A	ST	AdB	5-10 fold in 3 wk	+	+	Jelaska and Sutina 1977
	A	NS	AdB, AxB	12.8 fold in 6 wk	+	99.4	Roest and Bokelmanr 1981
icentra spectabilis	A	ST	AxB	50 fold in 6 wk	100	100	Lazarz et al 1982
ionaea muscipula	A, S	AB, LS	AxB, AdB	15.4 fold in 8 wk	+	+	Parliman et al 198 a, b

1	2	3	4	5	6	7	8
Dracaena deremensis	A	SS	C	+	+	+	Debergh 1976
Dracaena fragrans	A	NS	AxB	+	+	+	Debergh 1976
Dracaena godseffiana	A	ST	AxB, C	300,000 fold in 52 wk	+	+	Miller and Murash. 1976
Dracaena marginata	A	NS	C	+	+	+	Chua et al. 1981
Epiphyllum chrysocardium	A	NS	AxB	+	+	+	Lazarte et al. 198
Episcia cupreata	A	LS	C	20-30 fold in 6-8 wk	+	98	Johnson 1978a
Episcia sp.	A	LS,PS, SS,ST	AdB	+	+	80	Bilkey and McCown 1979
Eucalyptus ficifolia	S	NS	AxB	+	+	-	de Fossard et al. 1977
	A	NS	AxB	+	+	-	de Fossard et al. 1977
Euphorbia pulcherrima	NM	INS, PS	AdB, C	+	+	-	de Langhe et al. 1974
Ferocactus acanthodis s;	ST		AxB	+	+	90	Ault and Blackman 1987
Ficus lyrata	A	LS	C	+	100	100	Debergh and de Wae 1977
	-	NS in vitro	AxB	20 fold in 4 wk	100	100	Debergh and de Wae 1977,

1	2	3	4	5	6	7	8
reesia X hybrida	A	Pedicel	C	+	+	+	Stimart and Ascher 1982
reesia sp.	A	SS	AdB	+	+	+	Hussey 1977
uchsia hybrida	A	ST	C	+	+	+	Stevenson 1977b ar Harris 1980
erbera jamesonii	A	ST	AxB	+	+	+	Murashige et al. 1974
	A	Inf S	AxB AdB	3-4 fold in 12-16 wk	90	+	Pierik et al. 1975
ladiolus sp.	A	Inf.S,SS	AdB	+	77	-	Ziv et al. 1970
	A	AB	AxB	3-5 fold in 8 wk	+	+	Hussey 1977
revillea asmarinifolia	S	NS	AxB	+	85	+	Ben-Jaacov and Dax 1981
uzmania lingulata	S	S	AxB	+	+	100	Mekers 1977
ypsophila paniculata	A	ST	AxB,C	5-8 fold in 2 wk	92	+	Kusey et al. 1980
Haworthia planifolia	A	LS	C	+	+	+	Wessels et al. 197
Haworthia sp.	A	Inf. S	C	+	+	+	Kaul and Sabharwal 1972
Hemerocallis sp.	A	Petal segment	AdB,C	+	+	+	Heuser and Apps 1976
Hippeastrum spp.	A	FS	C	7 fold in 10 wk	+	+	Pajerski and Ascher 1982

1	2	3	4	5	6	7	8
	A	BS, LS, FS Ovary, Peduncle segment	AdB,C	450 fold (from one bulb) in 8 wk	+	+	Seabrook and Cumming 1977
Hosta decorata	A	ST	AxB AdB	+	100	100	Papachatzi et al. 1981
Hosta plantaginea	A	FB	C	+	+	1000	Papachatzi et al. 1980
	-	LS in vitro	AdB	+	+	+	Papachatzi et al. 1980
Hosta sieboldiana	A	Scape	C	+	+	+	Meyer 1980
Hyacinthus orientalis	A	BSS,LS,SS OD	AdB C	+	+	+	Hussey 1975
	A	FB,FBS	C	38-48 fold in 6 wk	+	90	Kim et al 1981
ydrangea uercifolia	A	NS	AxB	6 fold in 6 wk	+	+	Sebastian and Heuser 1987
lex aquifolium	A	Embryo	SE	+	+	-	Hu and Sussex 197
mpatiens ybrids	ST	AxB	+	+	NM	+	Han and Stephens 1987
ris hollandica	A	Inf S	AdB	+	+	+	Hussey 1976 b
ris sp.	A	Peduncle section	C	+	+	+	Meyer et al. 1975
	A	ST	SE	+	+	-	Reuther 1977
alanchoe ossfeldiana	A	ST	C	60-100 fold in 4 wk	+	99	Smith and Nightingale 1979

1	2	3	4	5	6	7	8
	A	SS	C	35 fold in 4 wk	+	99	Smith and Nightingale 1979
	A	LS	C	60 fold in 4 wk	+	99	Smith and Nightingale 1979
Lilium japonicum	A	LS	AdB	+	+	+	Niimi and Onozawa 1979
Lilium longiflorum	A	BS	AdB	18 fold in 4-5 wk	+	+	Gupta et al 1978
	A	BS	AdB	8-10 fold in 6 wk	+	+	Stimart and Asche 1978
Lilium pyrenacium	A	SS	AxB, AdB	10-20 fold in 5 wk	+	+	Hussey 1977
Lilium rubellum	A	LS	AdB	+	+	80	Niimi and Onozawa 1979
Lilium speciosum	A	BS	AdB	+	+	+	Robb 1957
	A	BS	AdB	+	+	+	Van Aartrijk and Blom - Barnhoorn 1981
Lilium sp.	A	BS	C	+	+	+	Simmonds and Cumming 1976
	A	BS	C	2.2 fold in 8 wk	+	+	Stimart et al. 198
	A	BS, ovary	AdB, C	10^4 - 10^6 fold (from one bulb) in 52 wk	+	+	Novak and Patru 1981
Lilium auratum X L. rubellum	A	LS	AdB	n +	+	+	Niimi and Onozawa 1979

1	2	3	4	5	6	7	8
Macropidia fuliginosa	A	AB	AxB	+	+	+	Ellyard 1978
Magnolia soulangeana	A	NS	AxB	6 fold in 4 wk	85	85	Maena (Pers. Com
Malus hybrid *Dainty*	A	ST	NM	4 fold in 4 wk	+	NM	Norton and Boe 1982
Malus hybrid *Golden Hornet*	A	ST	NM	4 fold in 4 wk	+	+	Norton and Boe 1982
Mammilaria elongata	A	Tubercle	C	+	100	100	Johnson and Eminc 1979
Monstera deliciosa	S	ST	AxB	8 fold in 6 wk	+	+	Fonnesbech and Fonnesbech 1980
Narcissus tazetta	A	TSS	AdB	+	+	80-90	Steinitz and Yahe 1982
Narcissus sp.	A	LBS	AdB	2,620 fold (from two leaves) in 21 wk	+	+	Seabrook et al. 1976
	A	LBS,FS, LS,OD	AdB	140 fold in 52 wk	+	+	Hosoki and Asahir 1980a
Nerine bowdenii	A	BS	AdB	+	+	+	Pierik and Ippel 1977
Nerine sarniensis	A	BS	AdB	+	+	+	Pierik and Ippel 1977
Nerine hybrids	A	BS	AdB	+	+	+	Pierik and Ippel 1977

1	2	3	4	5	6	7	8
ithogalum rsoides	A	BS,OS,sepal segment	AdB	+	+	+	Hussey 1976
	A	SS,LS	C	+	+	+	Hussey 1976
lis hedysaroides	A	NS	AxB	28 fold in 7 wk	+	+	Maene and Debergh 1981
argonium hortorum	A	AB,ST	AxB, AdB	+	+	+	Debergh and Maene 1977
argonium hybrid	A	ST	AdB	+	+	+	Cassells et al. 198
	A	PS	AdB	+	+	+	Cassells et al. 198
eromia scandens	-	LS in vitro	AdB	+	+	-	Kukutczanka et al. 1977
eromia viridis	A	LS	AdB	+	+	+	Hui and Zee 1981
eromia sp.	A	LS	AdB	>500 fold in 10 wk	+	+	Henny 1978
gularia minor= odoratissima	A	SS	C,SE	+	+	+	Prabhudesai and and Narayanaswamy 19
rbitis nil	S	AM	C	+	+	+	Bapat and Rao 1977
lodendron spp.	A	NS	AxB	15 fold in 9 wk	100	100	Debergh (Pers. Comm.)
ox drummondii	A	FB	C	+	+	+	Konar and Konar 1966
ox paniculata	A	NS	AxB,C	+	+	+	Schnabelrauch and Sink 1979
guicula anensis	A	LS	AdB	15 fold in 8 wk	+	+	Adams et al. 1979a

1	2	3	4	5	6	7	8
Polyanthus tuberosa	A	AB	+	+	+	+	Murlidhar and Meh 1982
Portulaca sp.	A	LS	AdB	+	+	+	Carlson and Skirv. 1978
Potentilla fruticosa cv. Coronation Triumph	A	ST	NM	26 fold in 4 wk	+	NM	Norton and Boe 1982
Potentilla fruticosa v. Sutter's gold	A	ST	NM	27 fold in 4 wk	+	NM	Norton and Boe 1982
rimula obconica	A	Inf S, FB	AdB,C	5 fold in 4 wk	+	100	Coumans et al. 197
runus cerasifera	S	ST	AxB	10 fold in 4 wk	100	100	Hammerschlag 1982
	A	ST	AdB	250 fold in 17 wk	+	92	Garland and Stoltz 1981
	A	ST	NM	6 fold in 4 wk	+	NM	Norton and Boe 198
runus tomentosa	A	ST	C	15 fold in 4 wk	+	NM	Norton and Boe 1982
yracantha coccinea	A	ST	C	11 fold in 4 wk	+	NM	Norton and Boe 1982
uesnelia uesneliana	A	AB	AdB	8 fold in 13 wk	+	+	Hosoki and Asahira 1980b
	-	LS in vitro	AdB	10.5 fold in 4 wk	+	+	Hosoki and Asahira 1980b
anunculus celeratus	A	Sepal, Petal, Anther	SE	+	+	+	Nataraja and Konar 1970

1	2	3	4	5	6	7	8
Rhododendron simsii	NM	ST	AxB	+	+	+	Preil and Engelhar 1977
Rhododendron sp.	S	ST	AxB	3.2 fold in 5 wk	+	+	Anderson 1975, 197
	A	ST	AxB	+	+	+	Kyte and Briggs 1979
	A	AB, ST	AxB	5.8 fold in 8 wk	+	+	Economou et al. 1981
	A	ST	AxB	3.4 fold in 6 wk	+	+	Hannapel et al. 1981
	A	FBS	AdB	+	+	+	Meyer 1981
hododendron sp.	A	ST	AxB	8 fold in 10 wk	85-90	+	Ma and Wang 1977
osa canina	A	AB, ST	AxB	+	65	+	Khosh - Khui and Sink 1982
osa damascena	A	AB, ST	AxB	+	76	+	Khosh - Khui and Sink 1982
osa hybrida	A	ST	AxB	3 fold in 5 wk	68	50	Skirvin and Chu 1979
osa hybrida	A	ST	AxB	3 fold in 8 wk	55	82	Hasegawa 1979
osa hybrida v. Bridal Pink	A	AB, ST	AxB	+	82	+	Khosh - Khui and Sink 1982
osa hybrida v. Tropicana	A	AB, ST	AxB	+	74	+	Khosh - Khui and Sink 1982

1	2	3	4	5	6	7	8
Rosa sp.	A	NS	AxB	3-5 fold in 4 wk	+	+	Davies 1980
Saintpaulia ionantha	NM	Leaf	AdB	30 fold in 8 6 wk	+	+	Cooke 1977
	A	PS	C,SE	5,000 fold (from one petiole) in 13-17 wk	+	80	Bilkey et al. 19
Salpiglossis sinuata	A	LS	C	20 fold in 14 wk	+	+	Lee et al. 1977
Scindapsus aureus	A	AB	AxB,C	100,000 fold in 52 wk	+	+	Miller and Murashige 1976
Sedium telephium	NM	LS	C	+	+	-	Brandao and Sale 1977
Sinningia speciosa	A	LS	AdB	+	+	+	Johnson 1978b
Spathiphyllum	A	SS	AdB,C	5 4 fold in 4 wk	+	+	Fonnesbech and Fonnesbech 1979
	A	AB	AdB,C	9 1 fold in 8 wk	+	+	Fonnesbech and Fonnesbech 1979
Spiraea x Bumalda	A	ST	C	38 fold in 4 wk	+	Nm	Norton and Boe 1982
Streptocarpus hybrids	A	Pedicel	AdB	300 fold(per pedicel) in 21-26 wk	+	100	Raman 1977
Syngoninm podophyllum	A	AB	AxB	5,000 fold in 52 wk	+	+	Miller and Murashige 1976
Tillandsia polystachya	S	S	AxB	+	+	100	Mekers 1977
Tulipa gesneriana	A	FS	AdB	+	+	NM	Wright et al. 19
Vriesea heliconioides	S	S	AxB	+	+	100	Mekers 1977

1	2	3	4	5	6	7	8
riesea poelmannii	A	AB	AdB	4 fold in 13 wk	+	+	Hosoki and Asahira 1980b
	-	LS in vitro	AdB	10 fold in 4 wk	+	+	Hosoki and Asahira 1980b
risea splendens	S	S	AxB	+	+	100	Mekers 1977
risea sp.	S	S	AxB	+	+	100	Mekers 1977

I(ii) Fruit and Nut Crops

Plant	Age	Explant	Mode of propagation	Rate of shoot multiplication	Rooting (%)	Transplantation success (%)	Reference
1	2	3	4	5	6	7	8
Acharus sapota	S	ST, other vegetative parts	SE	+	+	NM	Sachdeva and Meh 1983
Ananas comosus	A	ST	C	+	+	+	Mathews and Rang 1981
	A	AB	AxB	3 fold in 4 wk	+	+	Zepeda and Sagawa 1981
Ananas comosus interspecific hybrid	-	Seed	C	+	+	+	Srinivasa Rao et 1981
Carica heilbornii (C. pubescens X *C. stipulata)*	A	NS	AxB	38.4 fold in 6 wk	90	+	Cohen and Cooper 1982
Carica papaya	A	ST	AxB, C	7 fold in 3 wk	+	+	Litz and Conover 1978a
	S	Root segment	C	+	+	+	Chen et al. 1987
Castanea mollissima	S	NS	AxB	3 5 fold in 6 wk	80	80	Qi-guang et al 1986
Citrullus lanatus	S	ST	AxB	4-5 fold in 6 wk	+	+	Barnes 1979
Citrus aurantifolia	S	SS, LS	C	+	+	+	Chaturvedi and Sharma (see Chatu vedi 1979)

1	2	3	4	5	6	7	8
itrus aurantium	S	ST	AxB	+	80	80	Kitto and Young 19
itrus grandis	S	SS, LS	C	+	+	+	Chaturvedi and Mit 1974
itrus limania	S	NS	AxB, AdB	+	+	+	Barlass and Skene 1982
	S	INS	AdB	+	+	+	Barlass and Skene 1982
	A	NS	AxB, AdB	+	+	+	Barlass and Skene 1982
itrus madurensis	S	SS	C	+	+	+	Grinbalt 1972
itrus microcarpa	S	proembryo, immature seed	SE	+	–	–	Rangaswamy 1961
itrus reshni	S	NS	AxB, AdB	+	NM	–	Barlass and Skene 1982
	S	INS	AdB	+	NM	–	Barlass and Skene 1982
itrus sinensis	S	SL, LS	C	+	+	+	Chaturvedi and Mitra 1974
	S	STS	AdB	+	80	+	Barlass and Skene 1982
	S	NS	AxB, AdB	+	80	+	Barlass and Skene 1982
	S	INS	AdB	+	80	+	Barlass and Skene 1982

1	2	3	4	5	6	7	8
	A	STS	AdB	+	+	+	Barlass and Skene 1982
	A	NS	AxB, AdB	+	+	+	Barlass and Skene 1982
	S	STS	AdB		+	+	Barlass and Skene 1982
phomandra betaceae	A	AB	AxB, AdB	6-8 fold in 12 wk	+	+	Cohen and Elliot 1979
ospyros kaki	S	embryo, H S	C	$\overset{*}{+}$	+	-	Yokoyama and Taket 1976
ijoa sellowiana	S	NS	AxB	3 fold in 4 wk	94	74	Bhojwani et al. (unpublished data)
cus carica	A	AM	AxB	+	+	+	Meriithi et al 198
agaria ananassa	A	ST	AxB	+	+	+	Boxus et al. 1977
lus domestica	S, A	ST	AxB	30-50 fold in 8 wk	+	+	Lane 1978
lus sieboldii 'Calocarpa'	A	ST	AxB	5 fold in 5 wk	85	90	Singha 1982b
lus sylvestris	S	ST	AxB, AdB	6 fold in 6 wk	+	+	Abbott and Whiteley 1976
	A	ST	AxB, AdB	4 fold in 5 wk	80	+	Abbott and Whiteley 1976
26 rootstock)	A	ST	AxB	3-7 fold in 4 wk	97	85	Jones et al 1977
9 rootstock)	A	ST	AxB	+	85	+	James and Thurbon 1979, 1981

1	2	3	4	5	6	7	8
7 rootstock)	A	ST	AxB	13 fold in 4 wk	100	+	Werner and Boe 198
lus sp. MM 104; 106; MM 109	A	ST	AxB	5 fold in 4 wk	100	+	Snir and Erez 1980
lus sp. 'Almey'	A	ST	AxB	5 fold in 5 wk	95	90	Singha 1982b
lus sp. 'Hopa'	A	ST	AxB	5 fold in 5 wk	95	90	Singha 1982b
lus sp. cv. licious	A	AB	AxB	+	79	+	Srikandarajan et al 1982
lus sp. cv. nathan	A	AB	AxB	+	95	+	Srikandarajan et al 1982
lus X purpurea ley'	A	ST	AxB	5 fold in 5 wk	60	90	Singha 1982b
rus alba	S	LS, LS in vitro	AdB	+	+	-	Oka and Ohyama 1981
	S	HS	AdB	+	+	-	Oka and Ohyama 1982
rus indica	M	NS	AxB	+	+	-	Bapat et al. 1987
rus nigra	S	ST	AxB	3-5 fold in 3-4 wk	88 8	90	Ivanicka 1987
sa sp.	A	ST, inflorecence tip	AdB	+	+	+	Ma and Huang 1982
rsea americana	A	immature embryo	SE	+	+	-	Mooney and Van Staden 1987
oenix dactylifera	A	AB, ST	SE	+	+	+	Tisserat 1981
stacia vera	S	AB, ST	AxB, AdB	+	+	+	Alderson and Barghchi 1982

1	2	3	4	5	6	7	8
	A	AB, ST	AxB, AdB	+	+	+	Alderson and Barg chi 1982
	-	LS in vitro	AdB	+	+	+	Alderson and Barg chi 1982
Poncirus tryifoliata	S	NS	AxB, AdB	+	90	+	Barlass and Skene 1982
	S	INS	AdB	+	90	+	Barlass and Skene 1982
Prunus amygdalus	S	LS, CS, embryo	C	+	+	-	Mehra and Mehra 1974
	A	TT	AxB, C	+	+	-	Tabachnik and Kes 1977
Prunus avium	S	ST	AxB	4-15 fold in 8 wk	85	80	Jones and Hopgood 1979
Prunus avium	A	AB	AxB	+	100	+	Snir 1982a
Prunus insititia	S	ST	AxB	4-15 fold in 8 wk	90	90	Jones and Hopgood 1979
Prunus persica	A	ST	+	5-10 fold in [illegible] wk	-	-	Hammerschlag 1980
Prunus persica	A	ST	AxB	6 fold in 3 wk	90	60 8	Miller et al. 198
Prunus salicina	A	ST	AxB	10 fold in 3 wk	95	95	Rosati et al 198
Prunus X 'Hally Jolivette'	A	ST	AxB, AdB	+	+	+	Lineberger 1982
Prunus hybrid	A	ST	AxB, C	+	+	-	Tabachnik and Kes 1977

1	2	3	4	5	6	7	8
Punica granatum	A	NS	AxB	7-10 fold in 6 wk	85	80	Mascarenhas et al 1982
	A	LS	AdB	+	93	+	Omura et al 1987
Pyrus communis	A	ST	AxB,C	5 fold in 5 wk	80	+	Singha 1980
Pyrus serotina	A	ST	AxB	4-5 fold in 4 wk	58	74	Bhojwani et al. 19
Ribes nigrum	A	NS	AxB	+	+	+	Cohen et al. (Pers Comm)
Rubus fruticosus	A	ST	AxB	+	+	+	Zimmermann and Broome 1980b
Rubus idaeus	S	ST	AxB	6 fold in 4 wk	90	+	James et al. 1980
	A	ST	AxB	2-3 fold in 4 wk	+	+	Snir 1981
	A	ST	AxB,C	2 fold in 4 wk	64	90	Broome and Zimmerman 1978
	A	ST	AxB	4 3-5 fold in 4 wk	100	100	Welander 1985
Rubus sp.	A	AB,NS	AxB	6 fold in 4 wk	+	+	Cohen and Cooper (Pers. Comm.)
Rubus sp.	A	ST	AxB	+	+	+	Skirvin et al. 1981
Santalum acuminatum	S	NS	AxB	+	+	-	Barlass et al. 1980
	S	INS	AdB	+	+	-	Barlass et al. 1980
Santalum lanceolatum	S	NS	AxB	+	+	-	Barlass et al. 1980
	S	INS	AdB	+	+	-	Barlass et al 1980

1	2	3	4	5	6	7	8
accinium ashei	S	ST	AxB	5-15 fold in 13-17 wk	-	-	Lyrene 1980
	A	ST	AxB,AdB	+	-	-	Lyrene 1980
accinium Corymbosum	A	NS	AxB	5 fold in 6-8 wk	90-95	90-95	Cohen and Elliot 1979
accinium sp.	A	ST	AxB,AdB C	+	90	+	Zimmerman and Broome 1980a
itis vinifera	A	Ovule	SE	+	+	+	Mullins and Srinivasan 1976
	A	NS,PS Inf S,LS	SE	+	+	+	Krul and Worley 19
	A	NS	AxB	+	+	-	Jona and Webb 1978
	A	STS	AdB	+	+	+	Barlass and Skene 1980a,b
itis sp.	A	ST	AxB,C	10-35 fold in 6-7 wk	91	80	Chee and Pool 1982
	S	AM	AxB	+	+	+	Aldwincle et al. 1981

(iii) Beverage, condiment, Latex producing, oil seeds and vegetable crops

Plant	Age^{a}	Explant^{b}	Mode of propagation^{c}	Rate of shoot multiplication^{d,e}	Rooting (%)^{e}	Transplantation success (%)^{e}	Reference
1	2	3	4	5	6	7	8
llium cepa	S	radicle segment	C	+	+	-	Dunstan and Short 1978
	A	ST	AxB, AdB	3-5 fold in 4-5 wk	+	+	Hussey 1978b
	A	BS	AdB	10-20 fold in 8 wk	+	+	Hussey 1978b
Allium porrum	A	SS	C	3 fold in 8 wk	+	+	Debergh & Standaer de-Metsenaere 1976
	A	SS	C	72-120 fold in 6-8 wk	+	+	Dunstan and Short 1979
Allium sativum	A	ST	AxB, AdB	5-8 fold in 4 wk	100	85-90	Bhojwani 1980a
Allium tuberosum	S	LS	C	+	+	+	Zee et al. 1977
Ammi majus	S	HS	SE	+	+	23-26	Grewal et al. 1976
Apium graveolens	A	PS	SE	276 fold in 3 wk	+	+	Williams and Colli 1976
Araujia sericifera	A	NS, seed	AxB, C	+	+	+	Tideman and Hawker 1982
Armoracia lapathifolia	A	LS	AdB	+	+	+	Meyer and Milbrath 1977
Artocarpus heterophyllus	A	ST	AxB	+	+	-	Srinivasa Rao et a 1981

1	2	3	4	5	6	7	8
[illegible]	NM	[illegible]	C	5-12 fold in 4 wk	+	NM	Lee et al. 198
Asclepias rotundifolia	A	NS, STS	AxB, C	+	+	+	Tideman and Hawke 1982
Asparagus officinalis	A	AB, ST	AxB	+	+	25	Murashige et al. 1972
	A	AB, ST	AxB	+	+	95-100	Hasegawa et al. 19
	-	NS in vitro	C	2-6 fold in 4 wk	75	70	Yang and Clare 19
Beta vulgaris	S	ES	AxB	3-6 fold in 5 wk	+	+	Hussey and Hepher 1978
	-	LS in vitro, PS in vitro	AdB	3-6 fold in 5 wk	+	+	Hussey and Hepher 1978
Brassica alboglabra	S	HS, CS	C	+	+	+	Zee and Hui 1977
Brassica campestris	S	AB	AxB	+	+	+	Kuo and Tsay 1977
Brassica campestris	A	PS	SE	+	+	+	Bhattacharya and 1980
Brassica juncea	S	CS	C	6 fold in 5 wk	+	+	George and Rao 19
Brassica napus	A	FT	C	+	+	-	Walkey and Woolfit 1970
Brassica oleracea	S	RS, HS LS, CS	C	+	+	+	Bajaj and Nietsch 1975
	A	FB	AdB	3 5 fold in 5 wk	[illegible]	96	Anderson and Carstens 1977
	A	LS	AdB	+	+	+	Johnson and Mitch 1978

1	2	3	4	5	6	7	8
	A	LS	SE	+	+	-	Pareek and Chandr 1978
	A	ST	AxB	2 2-3.8 fold in 2 wk	+	+	Walkey et al. 198
Capsicum annuum	S	HS	C	+	+	+	Guney and Rao 197
	S	HS	C	+	+	+	Fari and Czako 19
Capsicum frutescens	S	CS	C	+	+	+	Guney and Rao 197
Carya illinoinensis	S	NS	AxB	+	75	+	Hansen and Lazart 1982
	-	immature embryo	SE	-	-	-	Merkle et al., 19
Coffea arabica	A	LS	SE	+	+	+	Sondahl and Sharp 1977
Coffea canephora	A	NS	SE	+	+	+	Staritsky 1970
Colocasia esculenta	A	ST	C,proto-corm like structures with roots	+	+	-	Abo El-Nil and Zettler 1976
	A	ST	C,proto-corm like structures with roots	+	+	+	Jackson et al. 197
Cucumis sativa	S	AB	AxB	+	+	+	Handley and Chambliss 1979
Cymbopogon spp.	-	Seed	C,SE	+	+	+	Jagadishchandra an Sreenath 1982

1	2	3	4	5	6	7	8
	A	Inf S	C,SE	+	+	+	Jagadishchandra ar Sreenath 1982
Daucus carota	-	Embryo	SE	+	+	+	Steward et al. 196
Dioscorea composita	A	NS	C	+	+	20-30	Datta and Datta 19
Elaeis guineensis	S	RS	SE	+	+	+	Barrett and Jones 1974
Elettaria cardamomum	S	S	C	+	+	+	Srinivasa Rao et a 1982
ucalyptus olybractea	S,A	NS	AxB	+	-	-	de Fossard et al. 1977
uphorbia lathyris	A	NS	AxB,C	+	+	+	Tideman and Hawke 1982
	NM	ST	NM	2 fold in 5 wk	+	+	Lee et al. 1982
uphorbia peplus	A	NS,INS,ST	AxB,C	+	+	+	Tideman and Hawker 1982
uphorbia tannensis	A	NS,seed	AxB,C	+	+	+	Tideman and Hawker 1982
pomoea batata	A	ST,AB	C	5 1-8 5 fold in 5 wk	+	+	Litz and Conover 1978b
actuca savita	A	ST,AB	AxB	+	80	85-90	Koevary et al. 197
	A	LS	AdB	+	80	85-90	Koevary et al. 197
ycopersicon sculentum	A	LS	C	+	+	+	Padmanabhan et al. 1974

	S	INS	C	+	+	+	de Langhe and de Bruijne 1976
	S	ST	AxB,C	+	+	+	Novak and Maskova 1979
lanihot utilissima	A	ST	AxB,AdB	+	+	+	Kartha et al. 1974
	A	ST,SS	AxB,C	+	+	+	Bajaj 1978
lea europaea	S	NS	AxB	6 fold in 5 wk	80	60	Rugini and Fontana 1981
riganum vulgare	A	LS	C	9,676 fold in 21 wk	100	+	Garland and Stoltz 1980
Pisum sativum	S	ES	C	+	75	+	Malmberg 1979
Rheum rhaponticum	A	ST,AB	AxB	2.8 fold in 2-3 wk	100	92	Walkey and Matthew 1979
	A	ST	AxB	5 fold in 4 wk	94	100	Roggemans and Clae 1979
Simmondsia chinensis	A	LS,SS	C	+	+	-	Rost and Hinchee 1980
	A	ST	AxB	+	+	-	Rost and Hinchee 1980
Solanum melongena	S	HS	C	+	+	+	Kamat and Rao 1978
	A	Pith	C	+	+	NM	Fossuliotis et al. 1981
Solanum tuberosum	A	TD	SE	+	+	+	Skirvin et al. 197

1	2	3	4	5	6	7	8
	A	NS	AxB	10 fold in 4 wk	100	+	Hussey and Stacey 1981
	A	ST	AxB,AdB	20.2 fold in 17 wk	+	+	Marani and Pisi 19
	-	NS in vitro	AxB,AdB	10 fold in 4 wk	95	+	Marani and Pisi 19
Vanilla planifolia	NM	AB	AxB	5 fold in 6 wk	+	NM	Cervera and Madrig 1981
Xanthosoma caracu	A	ST	C	1-3 fold in 8 wk	+	+	Strauss and Arditt 1980
Zingiber officinale	NM	AB	AxB,AdB	5.7 fold in 9-13 wk	+	+	Hosoki and Sagawa 1977

iv)Medicinal crops

Plant	Age[a]	Explant[b]	Mode of propagation[c]	Rate of shoot multiplication[d,e]	Rooting[e] (%)	Transplantation success[e] (%)	Reference
1	2	3	4	5	6	7	8
Agave fourcroydes	A	SS	C	10^6 fold in 52 wk	+	+	Madrigal et al 19
Agave sp.	A	seed segment	C	+	+	+	Groenewald et al. 1977
Aloe pretoriensis	A	seed segment	C	+	+	+	Groenewald et al. 1975
Angelica acutiloba	A	Pedicel	SE	+	+	+	Nakagawa et al. 19
Artemesia dracunculis	A	LS	AdB	+	+	+	Garland and Stoltz 1980
Atropa belladonna	A	RS	C	+	+	+	Chaturvedi 1979
Catharanthus roseus	NM	SS	C	+	+	+	Ramawat et al. 197
Chrysanthemum cinerariaefolium	A	Inf S	AxB, AdB	25 fold in 2 wk	+	+	Roest and Bokelman 1973
	NM	AB, ST	AxB, AdB	21.5 fold in 4 wk	+	+	Wambugu and Rangan 1981
Coptis japonica	A	Pedicel	SE	+	+	+	Nakagawa et al. 198
Digitalis lanata	S, A	ST	AxB	15-30 fold in 6 wk	100	85-98	Erdei et al. 1981

1	2	3	4	5	6	7	8
ioscorea alata	A	NS	AxB	+	+	+	Mantell [illegible]
ioscorea bulbifera	S	NS	AdB	+	100	-	Uduebo 1971
ioscorea floribunda	A	NS	AxB	+	100	+	Chaturvedi 1975
ioscorea rotundata	A	NS	AxB,C	65,000 fold in 25 wk	+	+	Mantell et al. 197
lycyrrhiza glabra	A	AB	AxB,AdB	$3x10^{14}$ fold in 52 wk	+	+	Shah and Dalal 198
cimum gratissimum	A	NS	C	31,780 fold in 52 wk	+	75-90	Ahuja et al 1982
cimum viridi	A	NS	C	307,350 fold in 52 wk	+	75-90	Ahuja et al. 1982
cimum basilicum X kilmandscharicum	A	NS	C	348,330 fold in 52 wk	+	75-90	Ahuja et al. 1982
cimum viridi X gratissimum	A	NS	C	122,940 in 52 wk	+	75-90	Ahuja et al. 1982
auwolffia erpentina	A	LS	SE	+	+	+	Mitra and Chaturve 1970
olamum surattense	A	LS, INS, Thin epidermal peels	AdB,C	+	+	NM	Gupta and Chandra 1982
	-	RS in vitro	AdB,C	+	+	NM	Gupta and Chandra 1982
tevid rebaudiana	S	LS	C	+	+	+	Yang and Chang 197

I (v) Forest Tree Crops

Plant	Age^a	Explant^b	Mode of propagation^c	Rate of shoot multiplication^{d,e}	Rooting^e (%)	Transplantation success^e (%)	Reference
1	2	3	4	5	6	7	8
Acer rubrum	A	ST	AxB	5 fold in 6 wk	100	+	Welsh and Sink 19
Alnus glutinosa	S	NS	AxB	+	+	95	Garton et al 198
Broussonetia kazinoki	S	HS	C	+	+	-	Ohyama and Oka 197
Calophyllum inophyllum	S	NS	AxB	+	+	+	Rao and Lee 1982
Castanea sativa	S	Embryo axis	AxB	+	50	-	Vieitez and Vieite 1980
Cinchona ledgeriana	S	NS,ST	AxB	+	100	-	Krikorian et al 1982
Cinnamomum taiwaniana	NM	ST	AxB	+	+	NM	Ma and Chen 1978
Dalbergia latifolia	A	SS,RS	NM	+	80	+	Mascarenhas et al. 1982
Dalbergia sissoo	A	NS	AxB	4 fold in 5 wk	+	+	Mukhopadhyay (Pers Comm.)
Eucalyptus camaldulensis	A	Leaf segment	C,AxB & in vitro formed shoots	34 fold in 3 wk *	50	-	Muralidharan & Mascarenhas, 1987

1	2	3	4	5	6	7	8
Eucalyptus citriodora	A	Lignotuber	C	+	+	-	Aneja and Atal 19
	S,A	ST	AxB	5-8 fold in 2 wk	45-50	40-50	Gupta et al. 1981
	S	Embryo	SE	+	+	-	Muralidharan & Mascarenhas, 1987
Eucalyptus globulus	A	ST	NM	+	+	40-60	Mascarenhas et al 1982
Eucalyptus grandis	S	NS	AxB	+	+	+	Cresswell and Nit 1975
	S,A	NS	AxB	+	+	90	de Fossard et al. 1977
ucalyptus arginata	A	NS	AxB	3 fold in 4 wk	+	+	McComb and Bennett 1982
	A	Stamen	C	+	+	+	McComb and Bennett 1982
	S	NS	AxB	10 fold in 4 wk	50	90	McComb and Bennett 1982
ucalyptus ova-anglica	S	ST	AxB,C	+	[illegible]	+	Mehra-Palta 1982
	S	NS	AxB	5 fold in 3-4 wk	[illegible]	+	Mehra-Palta 1982
ucalyptus regnans	S,A	NS	AxB	+	-	-	de Fossard et al 1977
ucalyptus erreticornis	A	TB	NM	+	+	40-60	Mascarenhas et al 1982

1	2	3	4	5	6	7	8
Eugenia aquea	S	NS	AxB	+	+	+	Rao and Lee 1982
Eugenia cumini	S	NS	AxB	+	+	+	Rao and Lee 1982
Eugenia grandis	S	NS	AxB,C	6-7 fold in 6 wk	+	90	Lee and Rao 1982
	S	INS	C	+	+	+	Lee and Rao 1982
Eugenia jambos	S	NS	AxB,C	+	+	+	Rao and Lee 1982
Ficus religiosa	A	LS	C	+	+	70	Narayan and Jaisw 1986
Fragarea fragrans	S	NS	AxB,C	+	+	+	Rao and Lee 1982
Hevea brasielensis	A	TB	NM	+	60	+	Mascarenhas et al 1982
Populus alba	A	ST,AB	AxB,AdB	10 fold in 4 wk	80	90	Christie 1978
Populus euroamericana	S	ST,cambium tissue	C	+	80	+	Chalupa 1974
Populus flevo (P. deltiodes x *P. nigra)*	A	AB	AdB	10^6 fold in 52 wk	+	+	Whitehead and Gil 1977
Populus nigra	A	AB	AdB	10^6 fold in 52 wk	+	+	Whitehead and Gil 1977
Populus tremula	A	ST,AB	AxB,AdB	10 fold in 4 wk	80	90	Christie 1978
Populus tremuloides	A	AB	AxB,AdB	10 fold in 4 wk	80	90	Christie 1978
Populus yunnanensis	A	AB	AxB,AdB	10^6 fold in 52 wk	+	+	Whitehead and Gil 1977
Paulownia taiwaniana	NM	INS	NM	+	+	+	Fu 1978

1	2	3	4	5	6	7	8
aulownia taiwaniana	NM	INS	NM	+	+	+	Fu 1978
aulownia tomentosa	A	Ovule	SE	+	+	+	Radojevic 1979
antalum album	S	HS	SE	+	+	+	Rao and Bapat 1978
	A	NS, INS	SE	+	+	-	Lakshmi Sita et al 1979
alix matsudana alba	A	NS	AxB	4 fold in 4 wk	100	90	Bhojwani 1980b
alix viminalis	A	NS	AxB	+	+	+	Garton and Read 19
wietenia mohagoni	S	SS	C	+	-	-	Rao and Lee 1982
ectona grandis	S	ST	AxB, AdB	500 fold in 52 wk	80	75	Gupta et al 1980
	S	NS, HS	AxB, AdB	2-3 fold in 2-3 wk	80	75	Gupta et al 1980
	A	ST NS	AxB, AdB	+	60	66-100	Gupta et al 1980
amarindus indica	S	NS	AxB	6-8 fold in 7 wk	60	+	Mascarenhas et al 1982
	A	NS	AxB	4-5 fold in 7 wk	[illegible]	+	Mascarenhas et al 1982
	A	AB	AxB, AdB,	8 fold in [illegible] wk	100	+	Iyer 19[illegible]

for abbreviations see the following table

ABBREVIATIONS

[a] A	adult
S	seedling
[b] AB	Axillary bud
AM	apical meristem
BS	bulb scale
CS	cotelydon segment
ES	epicotyl segment
FB	floral bud
FBS	flower bud segment
FS	scapes/flower stalk
FT	flower tissue
HS	hypocotyl segment
INS	internodal segment
LBS	leaf base segment
LS	leaf segment
LS in vitro	leaf segment from in in vitro formed shoots
Inf.S	inflorescence segment
NS	nodal segment
NS in vitro	nodal segment from in in vitro formed shoots
OD	ovary disc
OS	ovary segment
PS	petiole segment
RS	root segment
RS in vitro	root segment from in vitro formed shoots
SS	shoot segment
SS in vitro	shoot segment from in vitro formed shoots
SS	shoot segment
SS in vitro	shoot segment from in vitro formed shoots
ST	shoot-tip
STS	shoot-tip segment
TD	tuber disc
TSS	twin scale segment
[c] ADB	adventitious bud formation
AxB	axillary branching
C	shoot differentiation from callus culture
SE	somatic embryogenesis
[d] wk	week
[e] +	achieved but percentage not clearly mentioned
-	not achieved
NM	not mentioned